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PARALYTIC RABIES IN LIVESTOCK.

By H. V. M. METIVIER, B.SC., M.R.C.V.S.,
Government Veterinary Surgeon, Trinidad, B.W.I.

History of the Disease in Trinidad.

IN 1925, in the month of July, it was reported that cattle—young heifers—turned out to graze in their owner's paddock at St. Anns, a suburb of Port of Spain, Trinidad, were dying from a peculiar disease in which the chief symptoms were salivating, marked constipation and a staggering gait followed by paralysis; further, all the animals died from the condition in spite of early attention by veterinary surgeons. It is believed that all the veterinary surgeons in the colony had an opportunity to see cases, and they were all agreed that the disease was a most puzzling one.

The grazing land was thoroughly searched for poisonous plants by the Senior Scientific Officers of the Department of Agriculture, and enquiries were made to find out if any new method of husbandry had been introduced recently by the owner in the care and feeding of the animals. Both these investigations gave negative results. It should be mentioned that two suspicious weeds were found, but when fed to livestock at different periods of their growth they produced no symptoms in any of the cattle.

Within a short time cases of the same disease began to occur in two Government pastures—the St. Anns and the Queen's Park savannahs—where dairy keepers of the Port of Spain area turned out their dairy cows and heifers. In the larger of the two pastures there were about 300 bovines grazing and in the smaller one nearly 40 head, including a few horses. A factor in the three areas was that all the animals attacked were out of doors day and night and that stock in stables in the immediate environment were not affected. As soon as cases began to occur on these public pastures the Government was advised to have all the stock removed by their owners to their homes, because it was obvious that something abnormal in their immediate environment on the savannahs was responsible for the condition, as stabled stock at no great distance remained quite healthy.

On the Queen's Park and St. Anns savannahs the total

number of animals that died from the disease amounted to 20 per cent., many of them at the owners' residences after removal from the savannah. Some of the animals left the park in a healthy condition but later developed the disease and died. It was remarkable that animals removed from the savannahs died, at periods varying up to a month from the date of their removal, while the other stock already stabled at home remained quite healthy, even although the animals moved in from the pasture were placed in close contact (adjoining stalls and sheds, etc.) with them. From the very earliest, therefore, it was possible to decide that this disease was not of a contagious nature.

An excellent opportunity was afforded to see cases at all stages of the disease on these public enclosures, and some of the owners assisted by handing over their affected animals for slaughter so that *post-mortem* examinations could be held on animals in which putrefaction had not set in. Here again, in most cases the symptoms could be summarised as staggering gait, isolation from rest of stock, salivation, and later, paralysis; the condition pointed definitely to a form of bulbar paralysis. The *post-mortem* examinations held in all cases were of a negative character. In most animals (bovines) there was just a slight inflammation of the mucous membrane of the abomasum; all the other visceral organs were quite healthy in appearance, but there was congestion of the brain and meninges in all cases.

The disease next occurred in another Government pasture one mile west of these savannahs, where again 15 to 20 per cent. of the stock became affected and died. The pasture was again cleared of all stock and, with the exception of those animals in the incubative stages of the disease that died when removed to their sheds or stalls, no further deaths occurred and the condition appeared to have died out.

Towards the end of 1925 and early in 1926 the disease made its appearance in Diego Martin, about four miles west of Port of Spain, cases occurring at River Estate (Government Experimental Cocoa Plantation), where a public savannah with a dairy bull is maintained for the peasantry. A few cases were also reported by peasants, especially among cattle that were tethered out to graze during the night as well as by day. Owners in all cases in this newly affected area were advised to have their animals removed from grazing land and fed on cut fodder, preferably that removed at some length from the ground and away from low-lying grounds. The disease appeared to subside and only a few sporadic cases were reported for the rest of the year.

In 1927 the disease reappeared in the Diego Martin area, and it was now reported that even a few stall-fed animals were suffering from it; the peasants have open sheds with just a roof over head for stabling their stock. A few cases were reported in St. James, just on the western outskirts of Port of Spain, and

also at Laventille, two miles east of Port of Spain. Cases were also seen in the Couva district—28 miles from Port of Spain—in a small village east of Spring Estate. In these districts owners were advised to stall-feed their animals and to avoid feeding fodder from swampy grounds. With the exception of a few cases reported from the Diego Martin district during the rainy season in 1928, the disease seemed to be dying out, when suddenly in 1929, just as the rainy season started, it flared up in the southern part of the colony—Siparia, 55 miles from Port of Spain, and it was there responsible for heavy losses in livestock. The disease has continued from this time in a regular manner in the colony, most cases occurring in the rainy season, with a marked fall in the incidence of the disease in the dry season and the India summer. The condition has spread in an irregular manner, there being at times a district free from the disease surrounded by a number of badly affected areas.

From Siparia the disease has spread south, north, east and west, but the irregularity was most pronounced at the end of 1929, the only two affected areas being St. Joseph in the north and Siparia-Fyzabad area in the south, these two areas being about 45 miles apart and the intervening country, where most of the Sugar Estates are situated with the greatest amount of livestock, remaining free from the condition. The disease subsequently occurred in all these areas, and it can safely be said that, with the exception of an area beyond the northern range in Trinidad, it has now been seen in all parts of the Colony (1934). In 1929, 1930 and 1931, the death rate averaged over 1,000 cases each year, and 90 per cent. of the animals affected were bovines; but horses, mules and donkeys are also affected and cases have been recorded in goats, sheep and pigs. Only two cases have been seen in dogs.

At the time of the Siparia outbreak a peculiar disease also appeared in human beings, in which paralysis was a marked symptom and the mortality 100 per cent. The two conditions seemed really to be quite different, because up to this time the animal disease was chiefly one in which bulbar paralysis was the chief symptom, whereas the human disease took the form of an ascending myelitis. Further, human beings handling animals and living in close contact with those suffering from the disease never developed the condition.

At this time, a full description of the disease in animals, as well as the *post-mortem* and laboratory findings, were forwarded to research workers on Botulism in America, and they reported that they were convinced that the disease was Botulism. In spite of this it was impossible to reproduce the disease from germ cultures of the organism in the laboratory, and the protection afforded by the imported antitoxin did not seem to stop the condition.

In 1930 and 1931 cases began to appear in animals in a form that resembled ascending myelitis, the animals being able to eat and drink, moving around as on a pivot, but with no signs of salivation; at times in the earlier stages there was even no constipation.

Pawan[1] in examining sections of the brain of a human case and of Capuchin monkeys inoculated with brain material from human cases had come across cell-inclusion bodies that looked like Negri bodies, and, in order to get more expert advice on the condition, materials were forwarded to Dr. Hurst[1] of the Lister Institute and Dr. Flexner of the Rockefeller Institution. The diagnosis sent back by both these workers was that the disease in man was rabies. Specimens from bovines were then examined and Negri bodies were discovered. Material was then forwarded from bovines to Drs. Hurst and Flexner and the diagnosis of rabies in animals was confirmed. Hurst pointed out that a similar condition which had existed in South America for a number of years had been described as paralytic rabies, in which the vector was unknown but suspected to be the bat. Material was subsequently sent to Dr. Andrews, Director of the Research Laboratory, Weybridge, Dr. Roux of the Pasteur Institute, and Professor Finzi of Milan, Italy, all of whom were agreed that the disease was rabies; both the two last-mentioned stated that the virus was identical with that which they had received from South America.

Distribution in Other Countries.

The paralytic form of rabies is now a well-known disease in South America, where it is known by several names, such as "Bovine Plague," "Peste das Caderas," "Mal de Caderas." It was first reported in the southern Brazilian State of Santa Catharina by Carini,[2] where it was confused with rinderpest—horses, at that time, were held to be immune—and large sums of money were spent on building a laboratory for the manufacture of anti-rinderpest serum and vaccine. Animals in affected areas were actually protected against this disease, but suddenly it began to occur in equines. In 1911, Carini was sent from San Paulo to investigate the disease, and he was the first to diagnose the condition as a peculiar form of rabies. He reported that the number of rabid dogs seen in affected districts did not appear to bear any relation to that of rabid cattle and horses, and that there must be another vector. He further stated that trustworthy individuals had assured him that they had seen bats attacking cattle on pastures in broad daylight and that these animals subsequently died of the disease. Carini was unable to obtain any bats for investigation purposes. A campaign against dogs was also undertaken with full police measures of protection,

but in spite of this cattle and horses still continued to die; after a number of years the practice of killing dogs was discontinued.

In 1913 the disease had spread to the German settlement of Blumenau and as, in spite of strict police measures against dogs, the death rate was high—30 per cent. of the cattle and 15 per cent. of the horses succumbing to the disease, two German veterinary workers were engaged from Europe (Haupt[3] in 1914 and Rehaag[3] in 1916-1918) to carry out further investigations. These workers confirmed the findings of Carini that the disease was rabies. It caused enormous losses among cattle, and in districts to which it was confined no cases of canine rabies were observed. In regard to its manner of spread and distribution of cases, they found that the disease occurred on both banks of an unfordable river; that more cases occurred in the vicinity of forests and fewer cases in densely populated areas—facts pointing to the absence of any connection with dogs or other terrestrial animals.

In Paraguay a similar disease which had existed for a number of years in cattle was first described as " enzoötic paraplegia " and later as " bovine mal de caderas." In certain districts 50 per cent. of the stock was affected and the death rate was 100 per cent. The plague was at first attributed by certain workers to a poisonous plant, *Ipomohea digitata,* and later to a form of hæmorrhagic septicæmia; it was believed that a similar condition in Brazil and Uruguay was due to the same cause. Migone, L. E.,[4] and Pena, R.,[4] investigating the disease in 1931 proved that " Mal de Caderas des bovides " in Paraguay is caused by a virus closely allied to the rabies virus, but differing somewhat in its virulence. Reference is made in their paper to the transmitting agent and they seemed to be of the opinion that the dog was responsible.

In 1930 a similar plague made its appearance in the northern portion of the province of Corientes in the Argentine. The disease was most marked in the rainy season and it was alleged that poisonous weeds in the pastures were responsible for the condition. It was subsequently investigated by Quiroga, S. S.,[5] Acosta, J. L.[5] and Rottgardt, A.,[5] who summarised their numerous experimental observations by pointing out that the disease known in the Province of Corientes under the name of "Mal de Caderas" was caused by a neuro-tropic virus, the most fundamental characters and properties of which coincided with those of the virus of rabies. They further pointed out that the results obtained in serum neutralisation and cross immunity tests carried out with their virus and the fixed virus of rabies were conclusively in favour of the identity of the two viruses.

Remlinger, P.,[6] and Bailly, J.,[6] in 1931, reviewed the literature dealing with epizoötic rabies in the States of Santa Catharina and Rio Grande de Sul of Southern Brazil, and concluded that

the disease was not rabies but pseudo-bulbar paralysis, " Maladie d'Aujeszky," and that Carini, Haupt and Rehaag were wrong in their diagnosis. This conclusion was based on the results of serological and cross immunity tests. These authors[7] later in the same year (1931), in a publication dealing with this disease in Brazil, Paraguay and other South American countries and reporting on their findings with the viruses they had received, came to the conclusion that bovine " Mal de Caderas " in Paraguay, Bolivia and the Argentine, " Peste das Cadeiras " in the State of Rio Grande de Sul, and rabies in Santa Catharina, Brazil, are one and the same disease; and, inasmuch as they are of the opinion that Paraguayan plague is the paralytic form of rabies, the same conclusion must be drawn regarding it also.

In the north of Brazil and in the Rupununi cattle-ranching districts of British Guiana ranchers have reported a similar disease causing a heavy mortality in their stock during the last two or three years. Brazilian veterinary surgeons working in both areas have reported to the ranchers in British Guiana that the disease is paralytic rabies and the condition identical with the plague of Santa Catharina and Rio Grande de Sul. From Venezuela information has also been received about a similar condition, but up to the present only one affected animal has been landed in this colony from this neighbouring republic, although a large number of animals are imported yearly from it. It can therefore be claimed that this form of rabies, which started in about 1908 in Southern Brazil, is now known in Corientes (northern Argentine), Paraguay, Bolivia, north and south Brazil, British Guiana, Venezuela and Trinidad.

Cause.

The Disease is caused by the virus of rabies and the findings in South American countries as well as in Trinidad have been confirmed by Hurst of the Lister Institute, London, Flexner of the Rockefeller Institute, New York, Andrews of the Ministry of Agriculture and Fisheries, London, Krause of Vienna, Finzi of Milan, and Remlinger and Bailly of the Pasteur Institute, Algiers. All of these studied material from Trinidad, and those who had also received material from countries in South America say that it and the Trinidad virus are the same. The diagnosis has been made not only on the result of changes in the central nervous system of affected animals—congestion, mononuclear perivasculitis, etc., the presence of negri bodies in conjunction with Van Gehuchten and Nelis lesions, and experimental animal inoculations, but also by the neutralisation of the virus by means of the serum of animals immunised with *virus fixe* of rabies and cross immunity tests. Moreover, the symptoms produced in laboratory animals by this virus and the period of incubation do not differ in any particular way from the classical symptoms observed after

inoculation with the virus of rabies—a fact which differentiates the virus from others of a similar nature, such as that of Aujeszky's and Borna's disease, in which the period of incubation is much shorter in the first case (three to five days in rabbits) and much longer in the second case (17 to 50 days), the duration of the disease being also much longer. Andrews,[8] working with the virus at Weybridge, England, has pointed out that although inoculated rabbits and guinea-pigs show symptoms that are indistinguishable from those of ordinary rabies, guinea-pigs are considerably more susceptible to the Trinidad virus than rabbits. Hurst[9] and Pawan,[9] have also pointed this out—and noted in both species that subcutaneous inoculation into the cheek is an effective method of transmitting the disease. The virus is present in the nervous system

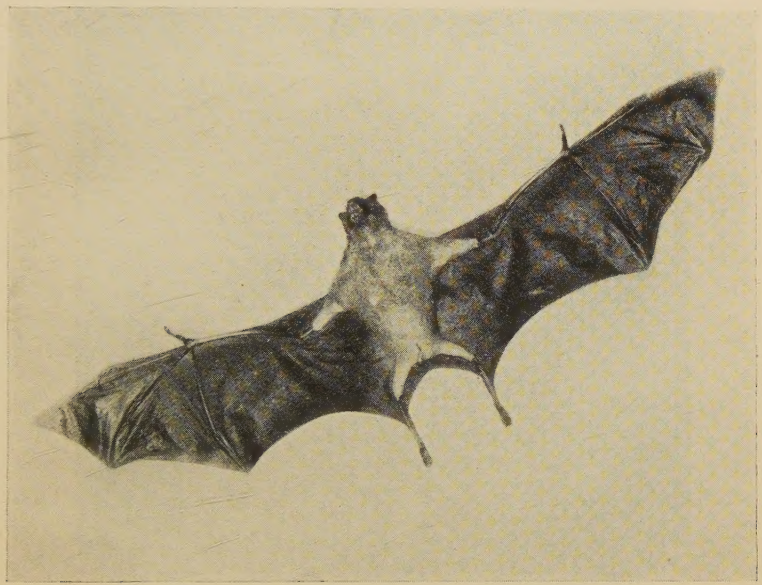

FIG. 1.

Vampire bat, *Desmodus rufus*. Stuffed specimen, 15½ inches across the wings.

(especially the brain) and the saliva of affected animals. It has not been found in the blood, but some workers in South America report its presence in the spleen and lymphatic glands, and experiments are being carried out with milk and urine to detect its presence.

Transmission.

In all countries rabies has been made the object of the strictest veterinary police measures for many years, these legislative measures being directed against canine rabies, which is by far the most important source of human infection. The incidence

of the disease in both bovines and equines is very small and would hardly justify the introduction of special laws in order to protect man if these animals alone were involved. In the South American plague there was a marked disproportion between the small number of rabid dogs and the large number of diseased cattle and equines, while in Trinidad there was never any connection between the disease in dogs and other animals. In Santa Catharina, in spite of the wholesale destruction of all stray dogs and prohibition of the importation of dogs, the disease still continued to spread. It was evident from the onset that there were some other means of transmission and, as cases were more pre-

FIG. 2.
Close-up view of Desmodus to show teeth.

valent in forest locations and rare in densely populated deforested districts, it was assumed that some wild animal was the transmitter. Certain insects and ticks were incriminated by others, but on investigation infection in this manner proved negative.

From extensive experiments it was recognised that the disease was not transmitted directly from one animal to another by contact. During their researches in South America, the attention of Carini, Haupt and Rehaag had been drawn to the bat—*Phyllostoma superciliatum*—as the vector, as this animal had been observed to behave in an abnormal manner in districts where the disease was occurring. Haupt and Rehaag later demonstrated

in Brazil that on the strength of both epidemiological and experimental data bats were the transmitting agents.

In Trinidad two species of bats received from affected areas were proved by experimental and histological examinations to be infected with rabies. It has been possible to infect not only laboratory animals but also bovines direct from the bat by the subcutaneous route. The main vector is *Desmodus rufus* or *rotundus*, the true vampire bat which feeds exclusively on mammalian and avian blood. It quickly approaches its host and makes an incision with its sharp upper incisors, lapping the blood as it flows from

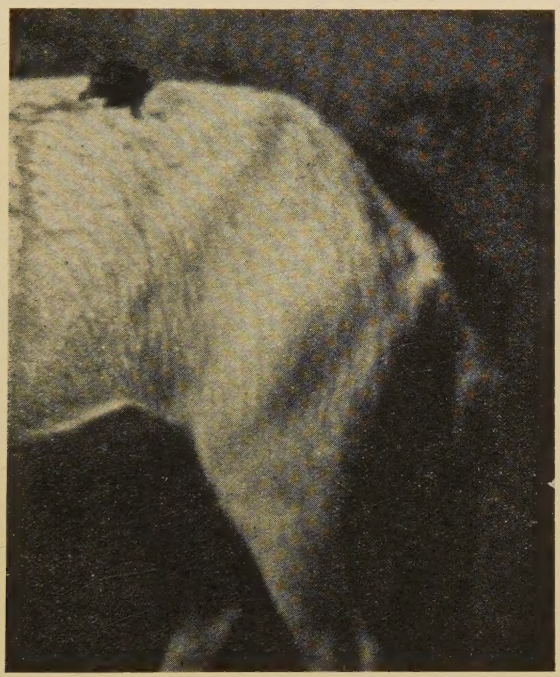

FIG. 3.
Bat on goat's back. Flashlight photograph.

the wound. The attack seems to cause the victim no discomfort and, while any portion of the body may be selected, the preferential site appears to be on the shoulders. Dark-skinned animals are more frequently attacked than are light-skinned ones and in the case of multi-coloured animals, such as Holsteins, the black areas are preferred to the white. Nevertheless bats may attack animals of any colour. The bats normally bite in the dark, especially in the evening, but if very hungry they attack in the dusk. The meal is digested during the night and the day is spent in resting. They often re-open old wounds rather than

make new ones. So far about 10 per cent. of all captured specimens have been found infected.

The fruit-eating bat, *Artibeus planirostris trinitatis*, has also been found infected naturally and rabid specimens have actually been seen fighting with each other.

Symptoms.

There is a great range of clinical symptoms but all animals eventually develop paralysis. Carini in the Santa Catharina plague and Migone and Pena in the Paraguayan outbreak claimed that there were two forms of the disease, (I) a quiet form, and (II) a furious form; in both an early symptom is incoördination of movement, especially of the hindquarters, and this is responsible for the name in Paraguay (*Mal de Caderas*), which confused it with trypanosomiasis. Haupt and Rehaag, on the other hand, state that they could not differentiate between a furious form and a paralytic one, and that the latter predominated in practically all cases observed by them; however, in a few cases, mildly pronounced symptoms of excitement, of short duration, preceded

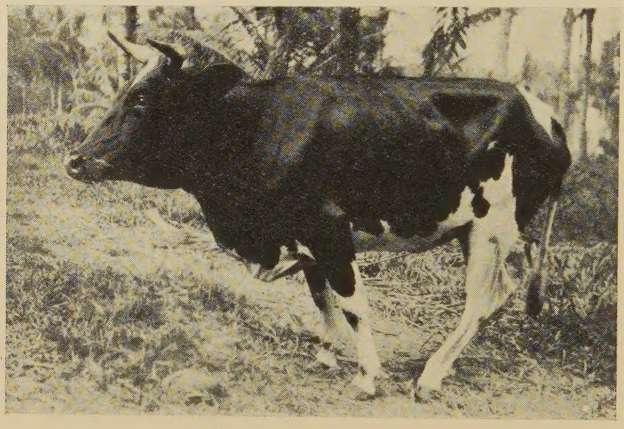

FIG. 4.
First day of illness.

the paralytic stage. The symptoms in Trinidad are more in accordance with the paralytic type, though in cattle, especially the Zebu (Indian humped) cattle, there is frequently a tendency for the animals to charge attendants and others, especially when on pasture. In equines, thoroughbred horses become most excited, bite themselves, gnawing a hole in their sides; a case was observed in a crooking mule where the animal actually bit its attendant on the leg.

The following symptoms of paralytic rabies were observed in cattle living under herd conditions and kept in a large paddock.

The affected animal is first observed to be grazing slowly apart from the herd in the northern portion of the pasture. A closer examination of the animal reveals symptoms of nervousness. There is an anxious expression about the face, and the ears move alternately forward and backward. When the animal is hurried forward there is noticed an involuntary, nervous, slow movement of the head from side to side, with frequent grinding of the teeth and swallowing motions; small quantities of saliva trickle from the mouth through tightly closed lips which twitch occasionally, with frequent upward contraction of the nose muscles. There is trembling of the muscles of the hind quarters and the animal's

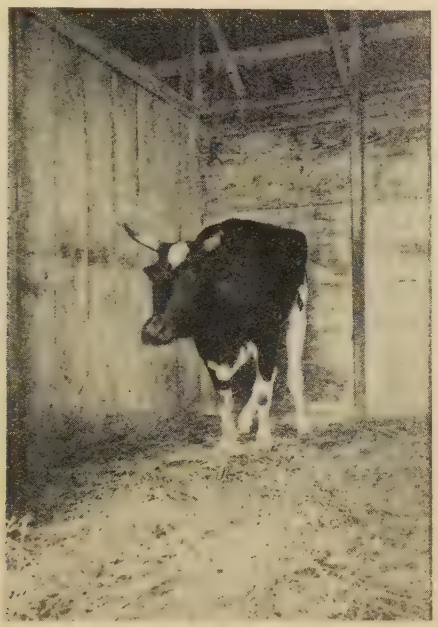

FIG. 5.
Same animal as previous figure, on second day.

gait reveals incoördination of movement in the hind limbs. If it is forced to cross a drain or increase its pace staggering of the hind quarters becomes pronounced.

On the second day the animal stops grazing, all the symptoms become more pronounced, and the inability to swallow causes profuse salivation. There is difficulty in rising from a recumbent position and the staggering gait of the hind quarters is most marked. If approached too closely the animal shows irritability and attempts to charge, even although it may previously have shown a gentle disposition.

On the third day the animal is unable to rise, and lies on one side with limbs moving occasionally; it salivates profusely and froths at the mouth and nostrils. At this stage urination ceases and the fæces have become very scant, hard, and formed in the shape of small marbles. The temperature is usually normal but the pulse rate is weak, small and fast. With the onset of paralysis consciousness is not at all disturbed; the half paralysed animals look distressingly behind, taking notice of everything that is happening around them and recognise their owners. There is hypersensitiveness of the paralysed parts at first, and this is later followed by complete anæsthesia; just before death there is complete skin anæsthesia over the whole body.

The animal usually dies in from three to five days after the first symptoms are noticed. In has been observed that the cattle of the Indian breed live much longer than the more delicate

Fig. 6.
Third day of illness.

European breeds (such as the Friesian) and, because of their greater excitability, they appear to suffer more during the final stages; their breathing becomes very laboured, they groan and move their heads and limbs about almost ceaselessly, and have frequently to be destroyed.

In about 2 per cent. of the cases the paralysis begins in the front limbs instead of the hind quarters.

A slight variation of the symptoms as above described has been observed in the early stages of the disease. In this there occurs a knuckling over of the fetlock joints of both hind limbs, becoming progressively worse until the hind legs keep slipping under the body towards the front legs, although the animal makes continual efforts to keep the hind legs in their normal position. The front legs meanwhile appear to be quite strong and bear most of the

body, until finally the animal lies down at about the second or third day; from then onward the symptoms of the two forms are indistinguishable.

In horsekind the excitement is more marked at first and there is frequently impaired vision; one case was seen in which the animal appeared to recover, but became totally blind and died after about six weeks. Another case that did not last quite as long showed symptoms of amaurosis when it appeared to be recovering. In other respects the disease is similar to that seen in bovines.

In pigs, sheep and goats, fewer cases have been observed, but they are typically of the paralytic form, while in the two cases seen in dogs the symptoms were identical with those of dumb rabies.

FIG. 7.
Shows bat bites on neck.

Pathology.

The macroscopic lesions were practically negligible in all cases examined, including several animals that were slaughtered when death appeared imminent. The chief lesions are congestion of the brain and meninges, the pia mater and the choroid plexus being invariably congested. In some cases there was a softening of the spinal cord in the lumbar region. The fullness of the urinary bladder and gall bladder and the frequent presence of hard pellets of fæces in the rectum (which is sometimes inflamed) are due to the paralysis, which was responsible also for some secondary changes in the intestinal tract.

In the brain there is a perivascular infiltration of the capillaries by round cells and lymphocytes, and the nerve cells show a granular degeneration and vacuolated nuclei. In sections of the hippocampus (Mann's stain) large numbers of Negri bodies are

frequently seen but, as Remlinger and Bailly have pointed out, these are not always present and this is true also of the ganglionic lesions of Van Gehuchten and Nelis.

Diagnosis.

The disease can always be recognised in the later stages by the paralysis and the high death rates, and it is generally indicated when a number of deaths occur in the same locality with a similar history within a short period. It is remarkable however that, even in the very earliest stages, when animals show few symptoms beyond slight twitching of the muscles of the body, lips and ears, some owners recognise the condition provided they have had previous experience of the disease. The normal temperature, the constipation, which seldom responds even to the most drastic purgatives, and the incoördination of movement terminating in paralysis, help considerably in arriving at a correct diagnosis. The disease is less easily diagnosed in the horse than in cattle. In the early stages owners sometimes mistake it for colic or tetanus —due no doubt to the symptoms of anxiety and excitement. As already indicated very little assistance can be obtained from macroscopic lesions at autopsy examination and in cases of doubt one resorts to microscopic examination of sections of the brain, and when that fails animal inoculation is necessary. In the field, it is difficult to transmit the disease from one animal to the other by subcutaneous or intramuscular routes, and these, as well as intraocular inoculation, have failed in all cases in the dog. The disease, as a rule, can easily be transmitted in series to rabbits and guinea-pigs either subcutaneously, intramuscularly in the jaw, or by the subdural route. After a series of passages in this way, it can be carried on in calves, and even in dogs.

Treatment.

Curative.—There is no curative treatment. Symptomatic treatment was carried out when the disease first made its appearance but was of no avail. The results were similiar in South American countries.

Preventive.—The control of the disease cannot be achieved by the eradication of the carriers, since bats cannot be destroyed easily in numbers. The best protection is afforded by the construction of " bat-proof " cowsheds and stables, in which animals should be confined at dusk and during the night. Among thousands of cases seen in Trinidad only one case was reported in an animal that slept in a bat-proof enclosure, but there have been numerous reports of cases occurring in animals that had not been bitten recently by bats.

Another preventive measure to repel the bats is *bright* illumination of sheds at night, but this can only be practised where a good electric light service is available and that is only possible

in towns, in which the incidence of the disease is negligible. On some large Sugar Estates, however, illumination is carried out as well as protective inoculation.

The vaccination of animals is the most suitable method of control in Trinidad as, in view of the cost, it is almost impossible for the peasantry to " bat-proof " their enclosures, and the lighting effect of kerosene lamps, etc., is of little use; moreover, many of their animals are not housed. Under ranching conditions in South America protective inoculation has given good results.

A committee was appointed by the Government of Trinidad to report on the disease in 1932 (the report being submitted in 1933), especially as to methods of control; this body strongly urged the protection of animals with rabies vaccine. From that date this work has been undertaken on a large scale and the disease has been proclaimed in order to compel owners to vaccinate their animals. In carrying out the campaign it was found that where steps were being taken to protect animals in advance of the disease owners objected on the ground that their stock was in good health and no deaths were occurring. As soon as the disease broke out in their district they demanded vaccination, with the result that frequently animals in the incubative stage of the disease died after vaccination and the vaccine got a bad name.

The vaccine in use is a carbolised one of fixed virus, using $33\frac{1}{3}$ per cent .of brain tissue in the emulsion. The results so far are very encouraging, and it has repeatedly been reported that owners who hid their stock during a vaccination campaign lost them from the disease. From South America (Argentine, Brazil, etc.), it has been reported by Quiroga[10] that vaccine (Kelser's method) composed of the local virus fixed through passage in the horse has given very good results, and another investigator (Costa[11]) reports very favourably on a vaccine of brain and serum (Fermi's method) from diseased animals. Further work along these lines is being carried out in London and in Trinidad.

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AU SUJET DE LA CLASSIFICATION DES PIROPLASMES DU BOEUF. LE GENRE *PIROPLASMA* ET SON SOUS-GENRE *BABESIELLA*.

Par Edmond Sergent, A. Donatien, L. Parrot et F. Lestoquard.
Institut Pasteur d'Algérie.

La classification des piroplasmes soulève encore bien des discussions. Il a paru utile de " faire le point " en ce qui concerne le genre *Piroplasma*.[1]

Le nom même du genre *Piroplasma* demande à être expliqué, et l'on ne peut mieux faire que de rappeler à ce sujet l'excellent commentaire qu'en fit F. Mesnil en 1925.[2]

Le nom qui, en réalité, a la priorité pour ce genre est *Hæmatococcus*, donné par Babès en 1888, pour désigner de petites formes endoglobulaires qui correspondent tout à fait à ce que nous appelons aujourd'hui une *Babesiella*. Mais Babès confondait sous le même nom des *Cocci* de cultures impures. De plus, *Hæmatococcus* désignait déjà une algue. Ce nom fut donc abandonné.

En 1893, Starcovici propose le nom de *Babesia*. Mais, comme l'ont fait remarquer Chalmers et Archibald, le nom de genre *Babesia* avait déjà été employé en 1889 par Trevisan pour désigner des bactéries. F. Mesnil estime qu'il est préférable de ne jamais employer deux fois le même nom de genre parmi les Protistes, même si l'un désigne un véritable protozoaire et l'autre un végétal ou une bactérie.

C'est pourquoi il se rallie au nom de genre *Piroplasma*, donné par Patton en 1895, et qui est universellement employé non seulement par les zoologistes, mais encore par les vétérinaires et les microbiologistes.

. . . . . .

L'étude qui suit ne vise que les espèces du genre *Piroplasma* qui parasitent les bovins, et qui ont fait l'objet d'expériences au laboratoire, à l'état d'infection pure. Ces espèces sont au nombre de cinq : *bigeminum, bovis, argentina, berbera, major*. Nous considèrerons ensuite la question de savoir si ces cinq espèces doivent rester groupées dans le même genre *Piroplasma* ou

[1]Nous avons déjà donné un aperçu de l'histoire et de la classification des *Babesiella* dans un mémoire des *Archives de l'Institut Pasteur d'Algérie*, t. IV, juin, 1926, pp. 318-339.

[2]*Bull. Soc. Path. exot.*, t. VIII, 14 avril, 1925, p. 243.

séparées en deux sous-genres : *Piroplasma sensu stricto* (comptant la première espèce : *P. bigeminum*), et *Babesiella,* Mesnil 1919 (renfermant les quatre autres espèces).

I. Caracteres Differentiels des Cinq Especes.

A.—*Caractères Spécifiques d'Ordre Morphologique.*

Les caractères morphologiques différentiels fondamentaux sont fournis par la taille des parasites et par la fréquence relative des petites formes rondes ou des grandes formes allongées. Le tableau ci-dessous donne les chiffres moyens :—

	Formes rondes.		*Formes longues.*	
	Pour cent.		Pour cent.	
Bovis	65	1μ	31	1μ 6 × 0μ 6
Argentina	72	1μ	18	2μ × 1μ
Berbera	72	1μ	24	1μ 8 × 1μ
Major	57	1μ 8	39	2μ 6 × 1μ 5
Bigeminum	3	3μ	85	3μ 5 × 2μ

Des caractères mineurs séparent les cinq espèces. Par exemple, l'espèce *bovis* occupe une place tout à fait à part par sa localisation fréquente sur le bord du globule rouge, qu'elle coiffe comme une calotte.

On peut noter aussi des différences marquées dans le nombre des parasites que l'on trouve d'ordinaire dans le sang de la circulation périphérique au moment des accès aigus :—

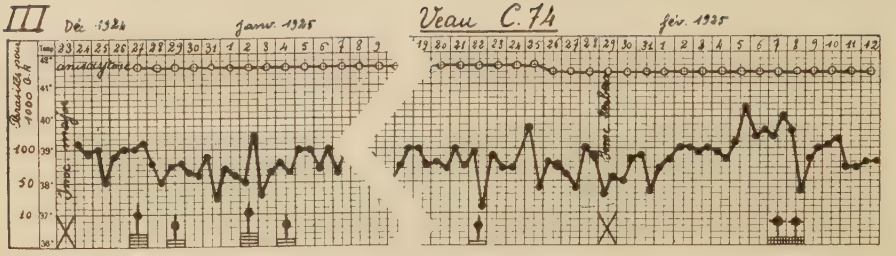
bovis	nombreux
argentina	rares
berbera	rares
major	assez nombreux
bigeminum	très nombreux

B.—*Caractères Spécifiques d'Ordre Biologique.*

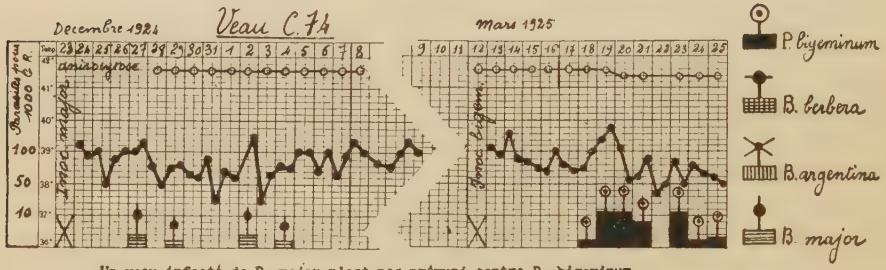
L'épreuve de la prémunition croisée (réaction de Laveran et Mesnil) quand ses résultats sont bilatéraux, nets et constants, met en lumière un caractère auquel nous attachons une valeur spécifique.

Le tableau suivant indique la présence (+) ou l'absence (O) de prémunition chez des veaux en état d'infection latente d'un virus, et que l'on inocule à nouveau, soit avec le même virus, soit avec un virus différent. (Voir les planches I, II, III).

III

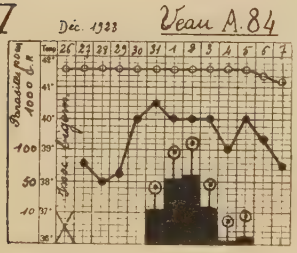


Un veau infecté de B. major n'est pas prémuni contre B. berbera.

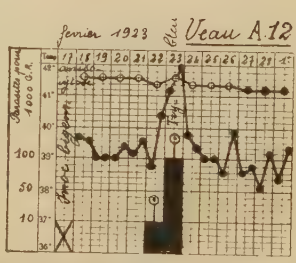


Un veau infecté de B. major n'est pas prémuni contre P. bigeminum .

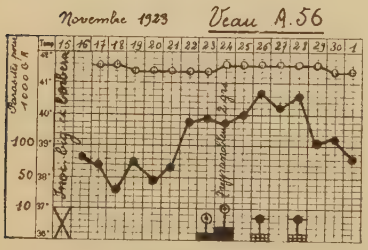
IV



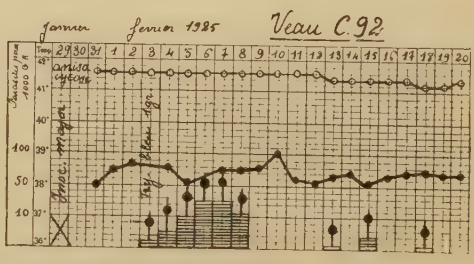
Accès normal de piroplasmose vraie à P. bigeminum.



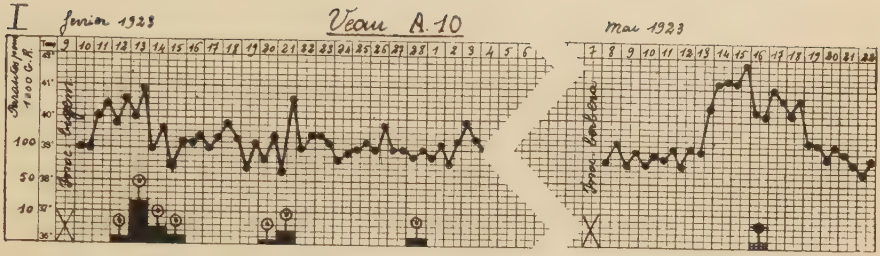
Accès de P. bigeminum coupé par le trypanobleu.



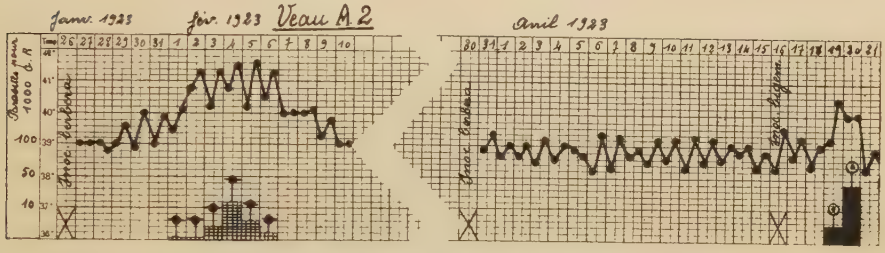
Efficacité du trypanobleu contre P. bigeminum
Inefficacité contre B. berbera .



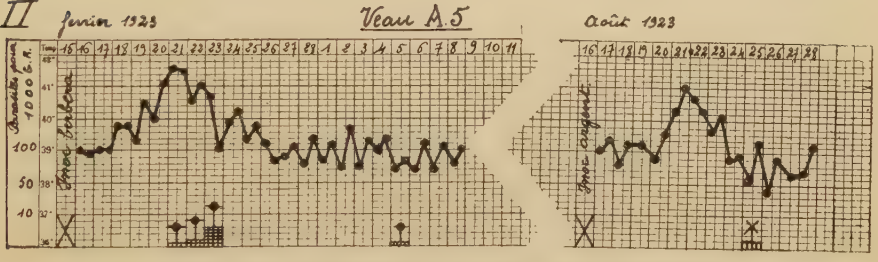
Inefficacité du trypanobleu contre B. major .



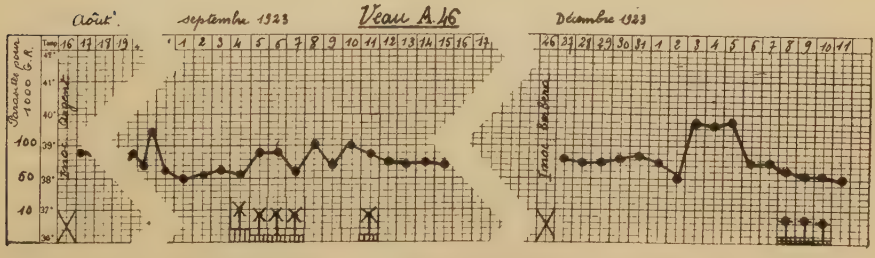
Un veau infecté de P. bigeminum n'est pas prémuni contre B. berbera .



Un veau infecté de B. berbera est prémmi contre une réinoculation de B. berbera ,
mais n'est pas prémmi contre P. bigeminum .



Un veau infecté de B. berbera n'est pas prémmi contre B. argentina .



Un veau infecté de B. argentina n'est pas prémmi contre B. berbera .

PLANCHES I, II, III.

Virus Inoculé.	*A des Veaux Infectés de :*				
	Bovis.	*Argentina.*	*Berbera.*	*Major.*	*Bigeminum.*
Bovis	+	O			
Argentina		+	O		
Berbera		O	+	O	O
Major			O	+	O
Bigeminum			O	O	+

II. REPARTITION DES CINQ ESPECES EN DEUX SOUS-GENRES.

Les différences qui séparent les deux types les plus anciennement connus, *bigeminum* et *bovis*, avaient été reconnues par M. Lühe en 1906[1] et par Du Toit en 1918.[2] Elles incitent F. Mesnil à proposer en 1919 de scinder le genre *Piroplasma* en deux sous-genres = *Piroplasma sensu stricto* pour les parasites qui occupent une place importante dans le globule rouge (type *P. bigeminum* Smith et Kilborne 1893), et *Babesiella* pour les parasites qui n'occupent qu'une petite place dans le globule rouge (type *B. bovis* Babès 1888).[3]

Depuis lors, on a décrit une espèce nouvelle = *major*, qui par sa taille, est intermédiaire entre les petites formes et le type *bigeminum*.

Nous estimons que la division en deux sous-genres doit être conservée, pour des raisons d'ordre morphologique et d'ordre biologique.

A.—*Caractères Subgénériques d'Ordre Morphologique.*

La différence est surtout dans la taille : les *Piroplasma* sont plus grands que le demi-diamètre du globule rouge du boeuf (qui est de 2μ 5 en moyenne), les *Babesiella* sont plus petits. Il s'ensuit que lorsqu'ils se divisent, les *Piroplasma* ne peuvent s'étirer en deux éléments disposés dans l'axe l'un de l'autre; ils sont obligés de se replier l'un sur l'autre d'où l'aspect classique de la " poire bigéminée," du " bissac." Au contraire les *Babesiella* se scindent en deux moitiés qui restent dans le prolongement l'une de l'autre d'où l'aspect en " haltère," en " boulets ramés." Un autre caractère qui coïncide avec la différence de taille est la différence de forme, allongée chez les *Piroplasma*, ronde chez les *Babesiella*.

[1] *In* Mense *Hdb. d. Tropenkrankheiten*, 1e-éd., III, 1906, 199.
[2] *Arch. f. Protistenk.*, XXXIX, août, 1918, 84-109, fig. ds le texte.
[3] *Bull. Inst. Pasteur*, XVII, 30 mars, 1919, 194.

B.—*Caractères Subgénériques d'Ordre Biologique.*

Un caractère d'une constance et d'une netteté remarquables est fourni par l'action d'un produit synthétique, le trypanobleu, sur les *Piroplasma,* et son inefficacité sur les *Babesiella.* Jamais nous n'avons constaté d'exception à cette règle. Il s'agit bien entendu d'expériences faites au laboratoire ou sur le terrain avec des animaux porteurs d'infection pure. Les expériences sur des animaux porteurs d'infections mixtes n'ont aucune valeur. Nous attachons une importance de premier ordre à cette preuve expérimentale, conformément au vieil adage: *naturam morborum curationes ostendunt.* (Voir la planche IV.)

PLANCHE IV.

Le trypanobleu injecté dans les veines, à un bovin, à la dose de 0 gr. 10 pour 100 kilogrs. d'animal, au cours d'un accès aigu, thermique et parasitaire, de piroplasmose vraie à *P. bigeminum,* provoque en quelques heures la chute de la fièvre et la disparition des parasites de la circulation périphérique. Les parasites ne reparaissent que tardivement et en petit nombre dans le sang. Le médicament a " coupé " brusquement l'accès et conféré du coup la prémunition au bovin.

Le trypanobleu possède la même activité contre toutes les espèces du genre *Piroplasma* parasitant d'autres animaux: *P. caballi* du cheval, *P. ovis* du mouton et de la chèvre, *P. canis* du chien.

Au contraire, il n'exerce aucune action sur le cours d'un accès aigu de babésiellose. Action nulle contre *bovis* (=*divergens*) dans les expériences de M'Fadyean et Stockman en Angleterre, action nulle contre *berbera* et contre *major,* dans les expériences de l'Institut Pasteur d'Algérie.[1]

. . . . . .

Le genre *Piroplasma* Patton 1895 comprend donc deux sous-genres et cinq espèces parasites des bovidés=

S.g.—*Piroplasma sensu stricto.*

Dimensions.—Plus d'un demi-diamètre de globule rouge (plus de 3μ en moyenne).

Forme prédominante.—Allongée = 85 pour cent. des parasites. Eléments en division pliés l'un sur l'autre, formant un angle aigu (poires jumelées).

Action souveraine du *trypanobleu.*

Une espèce:

P. bigeminum Smith et Kilborne 1893. Espèce ubiquitaire.

[1]Les faits contradictoires rapportés par Yakimoff sur l'action du trypanobleu contre *bigeminum* et contre *bovis* n'ont pas été confirmés à notre connaissance et devraient être vérifiés par des expériences sur des animaux en état d'infection pure.

S.g.—*Babesiella* Mesnil 1919.

Dimensions.—Moins d'un demi-diamètre de globule rouge en moyenne (2μ 5).

Forme prédominante = arrondie (de 57 à 72 pour cent. des parasites suivant les espèces). Eléments en division en paire d'anneaux, les deux parasites formant habituellement un angle obtus.

Action nulle du *trypanobleu*.

Quatre espèces :

La plus petite des *Babesiella*, remarquable par sa localisation sur le bord du globule rouge. = *B. bovis* Babès 1888 (= *divergens* McFadyean et Stockman 1911). Europe.

Deux espèces réunissant les éléments morphologiques moyens de *Babesiella* et ne se différenciant l'une de l'autre que par l'épreuve de la prémunition croisée = *B. argentina* Lignières 1911 (formes B et C de Lignières) causant la piroplasmose viscérale ou rénale des auteurs sud-américains.

B. berbera Edm. Sergent, Donatien, Parrot, Lestoquard, Plantureux, Rougebief, 1924. Afrique du Nord. Méditerranée. (? = *caucasica* Yakimoff et Belawine 1927. Caucase du Nord).

La plus grande des *Babesiella* =

B. major Edm. Sergent, Donatien, Parrot, Lestoquard, Plantureux, 1926. Europe. (? = *colchica* Yakimoff 1928, Caucase du Nord).

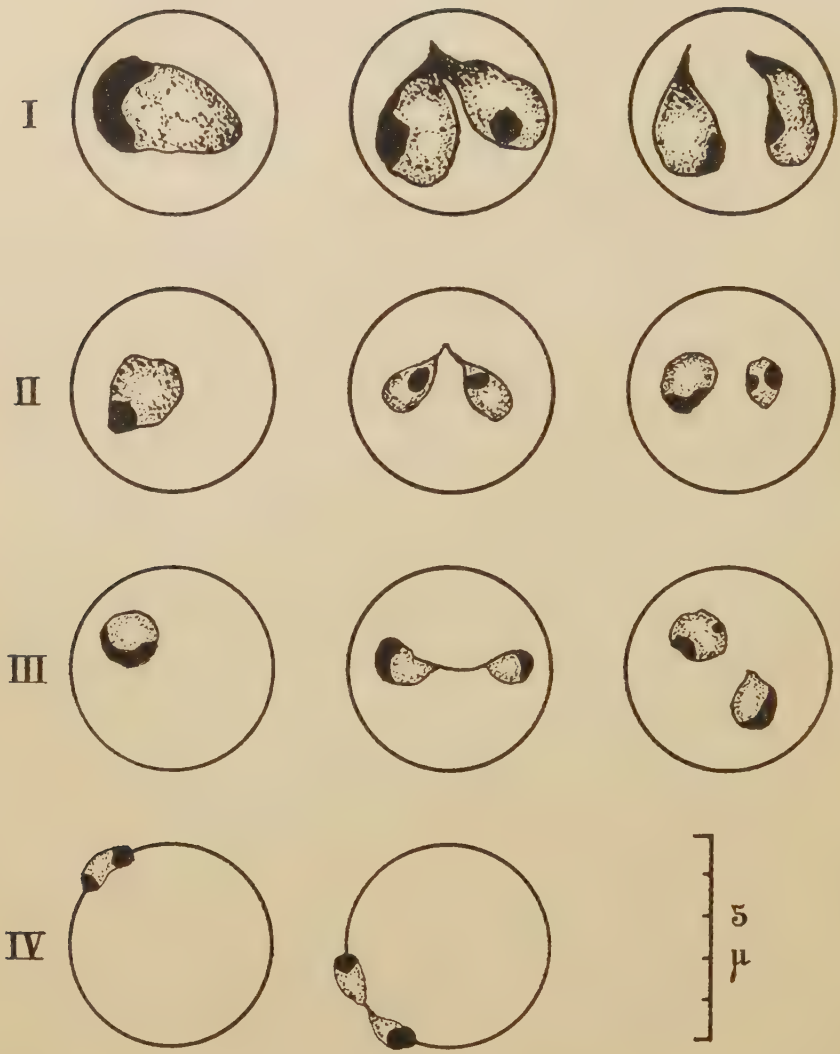


FIG. SCHEMATIQUE.

I.—Piroplasma bigeminum. II.—Babesiella major. III.—Babesiella argentina ou Babesiella berbera. IV.—Babesiella bovis.

DISEASES OF YOUNG CALVES: A BACTERIOLOGICAL EXAMINATION OF 100 CASES.

By Reginald Lovell and D. Leslie Hughes,

Research Institute in Animal Pathology, Royal Veterinary College, London.

Although losses among calves due to disease are known to be serious in this country there are at present no adequate statistics available to indicate how serious the losses are. It has recently been estimated by Jordan (1933) that in Ayrshire during 1931, of 548 calves born on 26 farms 113 (20 per cent.) died. The mortality among spring calves was distinctly higher than that among calves born in the autumn, the respective percentages being 26 and 8. One reason assigned for this difference is that cows calving in the autumn have had access to fresh green fodder during pregnancy. The figures, small as they are, give some index of the loss in one dairy county alone and suggest the need for investigation. It was, therefore, resolved in the first place to obtain for *post-mortem* examination the fresh carcases of not less than 100 calves dying within the first few months of life and to submit them to a detailed bacteriological examination. It was hoped in this way to provide a classification on an ætiological basis of diseases of young calves in this country. Naturally all diseases are not due to bacteria, and even the isolation of specific bacteria by no means completes the story. Errors in diet, housing and general hygiene may act alone or in concert with the prevailing bacteria flora, which although relatively harmless to adults may destroy newly-born calves. This point of view has been recently emphasised by Van Es (1930). Further, in a general way, and also in connection with the series of calves described below, it should be said that an ordinary routine technique would not reveal all species of bacteria present nor whether viruses played a part.

Technique.

Stockowners and veterinarians were asked to assist by forwarding carcases as soon after death as possible and by the quickest available route. Hence, it was possible in almost all cases to carry out the *post-mortem* examination within 24 or 36 hours of death. The issue of a questionnaire made it possible to correlate the *post-mortem* and bacteriological findings with such data as age, symptoms, duration of illness, methods of calf husbandry, and previous deaths. Calves showing *post-mortem* changes or giving the appearance of having been dead for more than 24 to 36 hours were not included.

The 100 calves examined were received from a wide area, 53 being supplied direct from 16 stockowners and 19 through eight veterinarians. A further 28 received from the Hannah Dairy Research Institute, had been purchased in connection with an experiment on nutrition.

Culture media were sown from the following sites : heart blood, lungs, mediastinal lymph glands, spleen, liver, bile, bone marrow (tibia), small and

large intestines and a gland associated with each. Cultures were also prepared from lesions seen in any situation. After searing the surface a portion of each organ was removed to a sterile petri dish. It was then cut into small pieces and emulsified with sand and about 3 c.c. of broth in a Griffith tube. With fluids such as blood or bile, the surface of the organ was seared and about one c.c. removed to 3 c.c. of broth. With bone marrow the tibia was sawn across and the cut surface seared before removing the marrow. The suspensions so prepared were diluted in Ringer's solution and used for sowing ox-blood agar deep plates. McConkey surface plates and tubes of serum broth were also sown from the original suspensions. After the first few calves had been examined the procedure was modified by making in addition glucose agar shake cultures from the lungs, mediastinal lymph glands, liver, spleen and bone-marrow. In some cases material from the lungs, mediastinal glands and spleen was injected intraperitoneally into mice, in order to obtain some indication of the pathogenicity of the bacteria present. All cultures were examined after 24 and 48 hours at 37° C. Colonies were picked from the plates and the organisms examined morphologically, biochemically and for pathogenicity ; in some cases also serologically. Finally, smears from the tissues mentioned were stained by Gram and portions of certain organs showing abnormality were preserved for histological study.

General Results.

It is proposed to set out the general results of the enquiry first and to follow with a short description of the special infections encountered. In assessing whether the organisms isolated played any part in the disease process, consideration was given to the site or sites from which the bacteria were isolated, and whether the bacterial species had been associated with calf disorders by previous authors. With some organisms the existence of

Table 1.

Findings in 100 *Calves Dying in First Few Months of Life.*

Single Infections.		
Bact. coli	37	
C. pyogenes	12	
Hæmolytic coccobacillus	6	
Salmonella	2	
Pasteurella	1	
	——	58
Mixed Infections.		
Bact. coli and *Ps. pyocyanea*	3	
Bact. coli, Pasteurella and *Staph. aureus* ...	2	
Bact. coli and *C. pyogenes*	1	
C. pyogenes and *Pasteurella*	2	
C. pyogenes and hæmolytic coccobacillus ...	2	
Pasteurella and *Staph. aureus*	1	
	——	11
Miscellaneous Group		31
		——
	Total	100

lesions at the site of isolation was taken to be of importance. Occasionally, it was difficult to decide into which class a certain case should be placed and it is, therefore, thought necessary to give as an appendix a brief description of each calf examined. The findings with the 100 calves are shown briefly in Table 1.

It will be noted from Table 1 that *Bact. coli* was isolated alone or in mixed culture from 43 per cent. of cases and that *C. pyogenes* was isolated alone or with other organisms from 17 per cent.

BACT. COLI *Infection.*

Although *Bact. coli* is a constant inhabitant of the intestinal tract of animals and man it is well known that under certain conditions it may be pathogenic. Whether *Bact. coli* normally present is identical with that isolated from disease processes will be discussed in later publications. It is sufficient at the moment to mention the early findings of C. O. Jensen (1893), Poels (1901) and Joest (1903) as authors who among others regarded *Bact. coli* as the most frequent cause of " white scours " in calves and other new-born animals. Among other organisms cited as the cause of this condition, Jensen mentions *Bact. aerogenes*, paracolon bacilli, *Proteus vulgaris*, whilst Poels cites in addition *Ps. pyocyanea*. It is rather difficult to bring the nomenclature used by these early authors into line with modern work as differentiation in the coliform group has advanced considerably in recent years. For instance, the " paracolon bacilli " of Jensen have been shown by J. Smith and Scott (1930) to be *S. enteritidis* var. dublin. Again, it is difficult to understand the meaning of " iso-coli " and " meta-coli," terms used by Danish authors (Mejlbo, 1934). In spite of such difficulties it is recognised that *Bact. coli* is responsible for much disease in young calves. Thus, Mejlbo (1934) found that in 1037 (20·6 per cent.) of 5,042 calves dying—mostly between the ages of two and ten days—" colibacillosis " was the cause of death. This condition has also been recognised in America by Theobald Smith and his collaborators (Th. Smith and Little, 1922A, 1922B, 1923, 1927, 1930 ; Th. Smith and Orcutt, 1925 ; Th. Smith and Bryant, 1927 ; Th. Smith, 1927, 1930 ; D. E. Smith, 1927). These papers give confirmation firstly to the fact that the chief infecting agent concerned with the high mortality in calves during the first week of life is some form of *Bact. coli*, the main symptoms and lesions being diarrhoea, in some cases inflammation of the umbilicus and multiple exudative arthritis. The authors suggest that special races of *Bact. coli* varying in their biochemical reactions may be associated with " white scours." Thus, in 1917 a motile saccharose-fermenting strain was incriminated whilst several years later a non-motile, non-saccharose-fermenting strain was implicated. It is also concluded that these special races of *Bact. coli* develop in calves which are weakly but that they are not virulent enough to affect stronger calves severely. Examination of a few strains isolated from the ileum of sick calves showed them to be pathogenic for calves intravenously, whilst filtrates injected by the same route occasioned acute respiratory distress. Mutation of certain strains was also studied, both serologically and biochemically. The papers also deal with the importance of colostrum to the new-born calf, the lack of this fluid or delay in obtaining it, permitting of the invasion of the body by intestinal organisms and their multiplication in various organs. Serum obtained from the mother or from a cow in the same herd also had some protective power, particularly if given intravenously and at the same time fed to the calf.

A general review of the 37 cases examined by us showed the age at which the calves died to vary from 24 hours to 16 days. Of these, 22 died within the first week of life and eleven during the second week. It should be made clear, however, that these proportions are probably not a true index of the age at which calves ordinarily die of *Bact. coli* infections, since the 37 cases included 19 specially selected for the Hannah Dairy Institute nutrition experiment. These 19 calves when purchased at two days old appeared to be strong and well. In the same connection the ages of eight calves recorded by Theobald Smith and Little (1922A) as dying of colibacillosis varied from two to eleven days, seven of the eight dying by the fifth day. The majority of the calves in Mejlbo's large series died between two and ten days of age.

It cannot be ascertained from our records whether sex has any bearing on the incidence of *Bact. coli* infection owing to the small number of calves and to the fact that bull calves only were bought for the Hannah Institute experiment.

Diarrhœa was stated to be a common symptom; with about half there was a history of inappetence, dullness and weakness, and in a few cases of respiratory distress.

According to the information supplied, all the calves in this series had access to colostrum, but it was of course impossible to obtain detailed information regarding the amount fed or the time of feeding.

At the time of *post-mortem* examination there was gross infection of the umbilicus in less than half the animals; enteritis was present in a similar number, but in only a few cases were the intestinal glands enlarged or otherwise altered. The lungs invariably showed some congestion and in a few cases macroscopic areas of necrosis. One may summarise by indicating the lack rather than the presence of any definite anatomical changes in calves dead from infection with *Bact. coli*.

There is no necessity in this communication to indicate more than on general lines the characters of the organisms isolated. They were all Gram-negative coliform rods, the majority being non-motile on isolation. Growth on ordinary culture media, including McConkey's agar, was good, and acid and gas were produced by all strains within 24 hours in glucose, maltose, mannite and lactose. The reaction with saccharose and salicin varied. All strains produced indol in peptone water, failed to liquefy gelatin, gave positive methyl red (M.R.) and Eijkman tests, and negative Voges-Proskauer (V.P.) tests, and failed to grow in Koser's citrate media. Litmus milk was acidified, bleached and sometimes clotted. Some strains were hæmolytic in blood agar.

The above characters are generally accepted as common to

strains of *Bact. coli* and organisms answering to the above description were isolated in accordance with the details in Table 2.

TABLE 2.

Sites from which Bact. coli *was Isolated in* 37 *Calves.*

Organ or fluid.	*Number positive.*
Blood	25
Lungs	32
Mediastinal glands	22
Spleen	28
Liver	26
Bile	20
Bone marrow	25
Intestinal glands	37

In five cases no cultures were made from the bile, and in three cases cultures from the blood, mediastinal glands and bone marrow were also omitted.

It is evident that in most cases the infection was of the nature of a septicæmia, particularly when attention is paid to the high proportion of positive cultures from the blood, spleen and bone marrow. As would be expected, the organisms were present in all cases in the intestinal contents and the intestinal glands.

C. PYOGENES *Infection.*

It is well known that *C. pyogenes* is implicated in many suppurative conditions of animals, *e.g.*, pneumonia in calves, so-called " summer mastitis " in cattle and in arthritic conditions in cattle, sheep and pigs. Of 12 strains studied by Brown and Orcutt (1920) four were from cases of calf pneumonia, whilst Theobald Smith (1925) isolated *C. pyogenes* from calves which had been born prematurely owing to *Br. abortus* infection and which showed lung lesions attributed primarily to infection with *Br. abortus*. Theobald Smith (1921) had previously isolated *C. pyogenes* from seven of twelve calves dead from broncho-pneumonia associated with *Actinobacillus actinoides* (*B. actinoides*). In Smith's view the latter organism produces necrotic foci, in which *C. pyogenes* and Pasteurella can multiply and cause more severe esions.

The ages of eleven of the twelve cases from which *C. pyogenes* alone was isolated varied from seven days to five and a half months, there being no exact record in one case. Seven of the calves were one month old or less, three were between one and three months old and one had lived for five and a half months.

The main symptom recorded was coughing, varying in persistence. One calf was said to have shown no symptoms till the morning of death, when food was refused; *post-mortem* examination, however, showed an advanced pneumonia. Two three-months-old calves had shown signs of pneumonia for a month before death, whilst two others had suffered from diarrhœa, inappetence and general weakness. There was no evidence in these two cases that *Bact. coli* was responsible for death.

The *post-mortem* findings were quite uniform, there being consolidation of the lungs, with areas of necrosis and suppuration one or more cm. in diameter. Sometimes these areas had softened to form a cavity. A fibrinous pleurisy with early adhesions was common, while blood-stained fluid was frequently present in the thoracic cavity and occasionally in the pericardial cavity. The mediastinal lymphatic glands were usually enlarged and hæmorrhagic. In two cases which had shown obvious signs of pneumonia for a month, there was a widespread invasion of the tissues with *C. pyogenes* (see Appendix, Nos. 7 and 8).

All the strains of *C. pyogenes* were typical in their characters, *e.g.*, in liquefying solid serum and serum gelatin, in clotting and digesting litmus milk, in producing hæmolytic colonies in blood agar after 48 hours' incubation. Acid was produced from glucose and lactose, and in some instances from other sugars such as maltose and saccharose. All strains belonged to one group by agglutination reactions. Corynebacteria with these characters were isolated from the lungs in all twelve cases and usually in pure culture. From six, *C. pyogenes* was also isolated from the mediastinal glands but from other sites only occasionally, apart from the two calves where there was widespread infection. So far, no special attempts have been made to isolate either *Br. abortus* or *A. actinoides* so it is not certain whether the *C. pyogenes* infections met with were primary or secondary.

An important point to be recorded is that seven of the twelve cases were received within a period of several months from one farm, suggesting the existence on this farm of a mild epizoötic.

Haemolytic coccobacillus *Infection.*

In a series of papers Jones (1921A, 1921B) and Jones and Little (1921, 1922) have described organisms isolated from pneumonia in cows and calves. In the two papers by Jones a description is given of 16 strains of "*B. bovisepticus*" which were found to fall into three biochemical groups. Eight strains were included in Group I, characterised by the production of slight acidity but no gas in glucose, maltose, mannite, lactose and sucrose, while salicin was unchanged. The strains were haemolytic, did not form indol and were insoluble in bile. Groups II and III were non-haemolytic and corresponded to Pasteurella, as commonly met with in animal infections. Jones and Little (1922) described an outbreak of rhinitis, complicated by pneumonia, among 32 calves due to "*B. bovisepticus*, Group I." Some of the calves died from a diffuse pneumonia whilst others recovered except for a somewhat persistent nasal catarrh. A similar organism could be isolated from the nasal exudate and was carried in the nasal passages for upwards of 121 days. It possessed little pathogenicity for laboratory animals, but undoubtedly was responsible for the catarrh and pneumonia. In this country Tweed and Edington (1930) have reported the isolation of a similar organism from pneumonia in cows and calves. On several farms the pneumonia was epizoötic and it is suggested that the disease was introduced by a carrier. Although strains hitherto named *B. bovisepticus* are usually accepted as belonging to the *Pasteurella* group, no general agreement appears to have been reached regarding the classification of the organism designated by Jones, "*B. bovisepticus* Group I."

We have encountered a similar organism and for the present refer to it as a "hæmolytic coccobacillus." Organisms of this class were isolated from six calves, from five days to two and a half months old, three being male and three female. Two of the calves were born prematurely and three were weakly at birth. The symptoms consisted of a cough with respiratory distress and nasal discharge. In one case there was a discharge from the ear. In all cases at *post-mortem* examination the lungs were deeply congested, with consolidation lobular in distribution. In several cases pus could be expressed from the bronchioles on squeezing the lung. In one case there were multiple small foci of pus but the change could be distinguished from that due to *C. pyogenes*, where the areas of suppuration were larger with occasional cavity formation. Pleurisy with adhesions, so common in *C. pyogenes* infection, was only apparent in one case and here there was also extensive necrosis of the fauces and necrotic ulcers on the tongue. Owing to the similarity between these lesions and those common in *F. necrophorus* infection an attempt was made to demonstrate this organism but without success. The intestines showed marked congestion in four cases and there was hæmorrhagic enlargement of the mediastinal lymphatic glands in three.

The strains isolated by us were Gram-negative coccobacilli, non-motile, growing readily on plain agar, producing grey discrete colonies with a smooth surface and an entire edge. In broth an even turbidity was produced. In deep blood agar plates after 24 hours there was a zone of hæmolysis, whilst on McConkey's agar tiny red colonies were produced. No indol was produced in peptone water. Acid was formed from glucose, maltose, mannite and sucrose; slowly from lactose, while salicin was not fermented. In order to show the acid production it was necessary to use brom-thymol-blue, since Andrade's indicator frequently failed to detect the slight degree of acidity produced. No change was observed in litmus milk. Pathogenicity tests in laboratory animals showed the virulence of the organisms to be low; five strains tested, using 1 c.c. of a 24-hour broth culture, failed to kill guinea-pigs by intraperitoneal injection, whilst half this dose by the same route killed mice in 24 to 48 hours.

The organisms were isolated from the lungs in all six cases. This was the only tissue from which they were isolated with any regularity, although in two calves blood cultures also were positive, while in one the organism was isolated from the liver, spleen and intestinal glands in addition.

That the organism is capable of causing an epizoötic has been shown by Jones and Little (1922) and Tweed and Edington (1930). Three of the calves in the present series belonged to a group of ten on one farm, all of which were showing symptoms of pneumonia with coryza and high temperature. Nasal swabs were examined bacteriologically from the remaining seven animals after

recovery and hæmolytic coccobacilli were isolated from two and *C. pyogenes* from a third. It was reported that the previous autumn a similar epizoötic had occurred on this farm, in a group of 17 calves following the introduction of a heifer calf which was slightly ill. We have no evidence of the actual cause of this earlier outbreak beyond the opinion expressed that the symptoms shown by the affected animals were similar.

Salmonella *Infection.*

Both *S. typhi-murium* (*Bact. ærtrycke*) and *S. enteritidis* var. dublin have been incriminated in diseases of calves. The disease described by Thomassen (1897) was probably of this nature and, as previously mentioned, the " paracolon bacillus " of Jensen, which causes the well-known " calf dysentery " of Denmark, is identical with the dublin variety of *S. enteritidis*. *S. enteritidis* or one of its varieties has also been connected with calf disease in America (Meyer, Traum and Roadhouse, 1916), Kenya (Daubney, 1927), Germany (Pröscholdt, 1931) and in Great Britain (Bosworth and Lovell, 1931). Henning (private communication) has recently examined a number of strains of Salmonella isolated from calves in South Africa and all but one were *S. enteritidis* var. dublin. Judging from the literature, this is the common Salmonella infection of calves and incidentally the same organism causes gastro-enteritis and continued fever in human beings. *S. typhi-murium* (*Bact. ærtrycke*) has also been recorded as causing disease in calves (Pröscholdt, 1931; E. O. Jordan, 1925), although less commonly than *S. enteritidis* var. dublin.

Salmonella bacteria were isolated from two calves in our series and in both cases the organisms belonged to the *S. typhi-murium* (*Bact. ærtrycke*) variety, although the strain isolated from the second case showed a lack of type antigen. Both animals were about one month old, the symptoms in one case being general weakness and diarrhœa, while in the other there was shivering, coryza and lacrymation for a week previous to death. In neither case were marked *post-mortem* lesions present, the second animal showing merely congestion of the lungs and small intestines. *S. typhi-murium,* identified by biochemical and serological methods (see Lovell, 1932), was isolated from the liver, spleen, intestinal contents and associated lymph glands in the first case and from the small intestine and intestinal glands of the second calf.

Pasteurella *Infection.*

Pasteurella organisms are commonly encountered in different species of animals and birds and it was customary to name them according to their animal source. It is now generally recognised that no reliance can be placed on the zoological origin of bacteria alone as a means of identification and in the case of Pasteurella this has been emphasised by Cornelius (1929) and Yusef (1935). For this reason we refrain from calling the strains now isolated

Pasteurella boviseptica. The designation "*B. bovisepticus*" used by Jones has already been referred to, and whilst it is probably incorrect to include the haemolytic variety (Group I) in the Pasteurella group, Groups II and III of Jones correspond to Pasteurella in their characteristics. Jones and Little (1922) record an outbreak in calves of a mild type of coryza associated with Pasteurella ("*B. bovisepticus*," Group II). The symptoms developed after those due to "*B. bovisepticus*," Group I had subsided and these authors were able to induce infection by nasal swabbing with cultures of both groups.

In only one case could Pasteurella alone be considered the cause of death. This was a 13-day-old female calf which had suffered from diarrhœa for a week. Although no mention had been made of any other symptom, *post-mortem* examination revealed great congestion and consolidation of the lungs, with pleural adhesions and blood-stained pleural fluid. A Gram-negative, non-motile coccobacillus was isolated in pure culture from the lungs and pleural fluid. This was non-hæmolytic, grew on agar, failed to grow on McConkey's medium and produced acid in glucose, mannite and saccharose. Indol was produced in peptone water. Litmus milk was not changed and the organism was highly pathogenic for mice by intraperitoneal inoculation. On these grounds it was identified as a pasteurella.

Mixed Infections.

Eleven cases were placed under this heading, since two or more organisms appeared to have played some part in the disease process. In five of the eleven cases the respiratory organs were involved and such organisms as *C. pyogenes, Pasteurella* and hæmolytic staphylococci (*Staph. aureus*) were isolated. These five calves are considered together, three of them being from the farm referred to above where the epizoötic due to *C. pyogenes* existed. The ages varied from nine to 31 days, symptoms of pneumonia, general weakness and lacrymation being common. *Post-mortem* lesions were largely confined to the thoracic cavity, and consisted of consolidation of the lungs, with purulent foci. The relevant details are given in the Appendix (Nos. F1, F10, 20, 38 and 51).

The remaining six cases coming under the heading of "mixed infections" might perhaps have been included under the *Bact. coli* infections, but as other potential pathogens were present these may have played a secondary part. Five of the six calves were eleven days of age or less, the other being one month old. The former had shown symptoms of "white scours," and the *post-mortem* findings were slight. In three of them *Ps. pyocyanea* was the secondary organism. The data are included in the Appendix (Nos. 53, 56, 63, 67, 71 and 85).

Miscellaneous Group.

Of the 100 calves examined, 31 fall into a miscellaneous group, in which the cause of death was variable. Whilst it is possible that some cases were due to bacterial infection, several certainly

died from other causes. For example, in one case there was extensive rupture of the abomasum, with peritonitis; whilst two, and possibly one other, appeared to have died from hydrocyanic acid poisoning. This condition is well known to veterinary toxicologists, the source of the hydrocyanic acid being linseed cake which when macerated with warm water may yield sufficient hydrocyanic acid to kill cattle fed with it. Hot water destroys the enzyme responsible for liberating the poison. Further details of this condition are given by Clough (1928), who emphasises the undesirability of feeding linseed cake which yields more than 0·02 per cent. of hydrocyanic acid when macerated in warm water. Dr. Clough has kindly informed us that a sample of cake similar to that fed to one and possibly to two calves yielded under these conditions 0·055 per cent. of hydrocyanic acid.

Another condition met with was a form of paralysis, particularly affecting the hind limbs. Two calves born in the same part of the country exhibited these symptoms, death following a period wherein the animal could not rise although still taking food and water. So far it has not been possible to throw any light on the ætiology of the condition, although it seems unlikely that any easily grown bacterial species is responsible.

Certain of the cases, however, were undoubtedly due to bacterial invasion, though it was impossible either to classify the bacteria concerned or to fit the case into any of the defined divisions. Thus from two calves streptococci were isolated from such sites as would suggest some connection with the disease process. In one case streptococci giving alpha-hæmolysis in blood agar plates were isolated from the blood, spleen, lungs and liver of a five-day-old calf which had died somewhat suddenly showing congestion of lungs and mediastinal glands only. A four-day-old calf showing similar *post-mortem* findings yielded alpha-hæmolytic streptococci from the lungs, liver, mediastinal glands and bile. In neither instance could the streptococci be identified with any of the varieties which have been isolated from mastitis by other workers in this Institute.

From eleven other calves there were isolated bacteria which may have had some connection with the disease process. Thus hæmolytic coliform bacteria which were atypical in some important characters were isolated from a two-day-old calf showing at *post-mortem* congestion of lungs and intestines, whilst from a nine-day-old calf showing similar anatomical changes at *post-mortem*, hæmolytic gelatin-liquefying coliform bacteria in addition to typical *Proteus vulgaris* were isolated. *Bact. coli*, *Bact. alkaligenes*, *Proteus vulgaris* and *Ps. pyocyanea* were isolated from others, but owing to the sites from which they were isolated—frequently in association with other organisms—it was considered preferable to adopt a conservative attitude and not to attempt a classification.

Discussion.

The object of this investigation was to survey the bacteriology of the diseases of calves in this country and to attempt classification on this basis. From the survey of 100 cases it is possible to point out varieties of diseases and to obtain some idea of their relative importance in Great Britain.

The greatest number of pure infections, *viz.*, 37 per cent., were due to *Bact. coli*, although actually *Bact. coli* was involved in 43 per cent. These infections were largely of the nature of " white scours," a disease known for many years to attack calves during their first few days of life. The results here recorded are in agreement with those of Jensen and his colleagues in Denmark and of Theobald Smith and his co-workers in America. Jensen (1893) investigated the disease which had been known in his country for 100 years and which on certain farms caused as high a death-rate as 66 per cent. He showed that the disease could be reproduced by feeding with fæces from diseased calves and also with pure cultures of the causal organism, which was identified as *Bact. coli*. Similar results were obtained by injuring the intestinal mucosa with chemical preparations and so allowing intestinal organisms to invade the body tissues. He believed that the organism was always present in cattle and that certain races of *Bact. coli* were potentially pathogenic for young calves. At a later date Jensen (1905) dealt with methods of prevention by serum treatment. His work indicated the most frequent route of infection to be by the mouth, although it was admitted that infection might occasionally enter by the umbilicus. On the other hand, Nocard and Mettam (1901), Mettam (1903), investigated diseases of calves in Ireland and concluded that two forms of disease existed, *viz.*, the typical white scours affecting calves of a few days old, and the so-called " lung disease " of older calves. They believed that both forms were due to Pasteurella and that, as infection was *via* the umbilicus, adequate attention to the umbilicus at parturition should effectually control the disease. The prophylactic measures advocated by them did not, however, materially reduce the mortality. The work of Nocard and Mettam has been adversely criticised in a series of editorial articles and annotations (*J. Comp. Path. & Therap.*, 1902A, 1902B, 1903), wherein is emphasised the lack of evidence of pasteurella as the cause of "white scours" and the probability that the views of Jensen were correct as regards the cause and common portal of entry of the coliform organism. Jensen's work has in fact been amply confirmed (Joest, 1903; Titze and Weichel, 1908; Jensen, 1911; Th. Smith and colleagues, *loc. cit.*). The fact that *Bact. coli* is responsible for a large proportion of deaths among calves during their first week or so of life is, therefore, well established, the typical syndrome being known as " white scours."

The respiratory infections in this series of calves were due to

C. pyogenes, a hæmolytic Gram-negative coccobacillus, and Pasteurella and these results also fall into line with the work of authors cited. It is probable that Nocard (1901) was dealing with mixed and pure infections of *C. pyogenes* in his work in Ireland. In cases of " lung disease " of older calves, he records finding a polymorphous bacillus which stained by Gram's method, but Pasteurella was considered to be the primary cause both of " white scours " and of " lung disease." In a few of our cases both Pasteurella and *C. pyogenes* were present, but there is no evidence that these organisms were secondary to white scours. All of these organisms are probably carried in the nasal passages of other bovines and the infection is apparently introduced by newly-purchased animals. They are, therefore, known to be associated with respiratory disease of calves, carriers probably playing their part in dissemination, but whether it is necessary for a primary focus to be laid down by other bacteria, such as *Br. abortus* or *A. actinoides,* is not at the moment clear. There is, however, evidence that in the case of *C. pyogenes* a prolonged illness may result in wide distribution of the organism throughout the animal body, pointing to an invasive capacity of this bacillus.

Of the 28 calves received from the Hannah Institute, 25 had been bought for a nutrition experiment; of these 14 had been fed on raw milk and eleven on pasteurised milk. From the lungs of four which had received raw milk *Staph. aureus* was isolated. These strains formed β toxin similar to that produced by most staphylococci from animals (Minett, private communication). Hæmolytic staphylococci were not isolated from those which had received pasteurised milk, and thus there may be some connection between the feeding of raw milk and the presence of staphylococci.

It is difficult to assess the significance of the bacteria isolated from the miscellaneous group of cases, but in 13 out of the 31 cases it is probable that some part at least was played by micro-organisms. The figures, therefore, of cases in which bacteria are estimated to have been responsible for death are 69 per cent. and, if the 13 of the miscellaneous group are included, 82 per cent.

Summary.

(1) One hundred calves dying within the first few months after birth were examined, and an attempt has been made to classify on an ætiological basis the diseases of calves occurring in this country.

(2) In 69 cases it was possible to isolate and identify the associated organism or organisms.

(3) Pure infections with *Bact. coli* accounted for 37 cases, *C. pyogenes* for 12, hæmolytic coccobacilli for six, *Salmonella typhi-murium* for two and Pasteurella for one.

(4) Mixed bacterial infections accounted for 11 cases, such

organisms as *Staph. aureus* and *Ps. pyocyanea* being present in addition to certain of those encountered in pure infections.

(5) In the remaining 31 cases the ætiology was doubtful, indicated causes not considered bacterial, or associated with bacteria which could not be identified with any known species.

We wish to record our thanks to the veterinary surgeons and stock-owners who rendered us assistance in making arrangements and in forwarding calves for our examination. Our especial thanks are due to Dr. Norman Wright, Director of the Hannah Dairy Research Institute, for sending calves and for supplying information.

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Appendix.

Brief details of each case dealt with.

Bact. coli *Infections* (37 *cases*).

F4 1 day. Hæmorrhages in spleen, with congested lungs. *Bact. coli* from spleen, lungs, intestinal glands.
F5 2 days. Mild congestion of intestines. *Bact. coli* from spleen, lungs, liver and intestinal glands.
F13 3 days. Congested lungs. *Bact. coli* from lungs, spleen and intestinal glands.
3 2 days. F. Diarrhoea, enteritis, *Bact. coli* from blood and all organs.
17 2 days. M. Slight congestion of organs, *Bact. coli* from blood and all organs.
21 7 days. F. Diarrhœa, slight congestion of lungs. *Bact. coli* from lungs, spleen and mediastinal glands.
24 2 days. M. Some congestion of lungs. *Bact. coli* from blood and all organs.
26 1 day. F. Respiratory symptoms, hæmorrhagic pleural fluid, *Bact. coli* from blood and all organs.
32 3 days. M. Inappetence, slight congestion of lungs, *Bact. coli* from lungs, marrow, mediastinal and intestinal glands.
37 4 days. F. Respiratory symptoms, congestion of lungs with necrotic foci. *Bact. coli* from blood and all organs.
42 2 days. F. Diarrhœa, exudate around umbilicus. *Bact. coli* from lungs and marrow.
43 5 days. M. Diarrhœa, exudate around umbilicus, *Bact. coli* from blood, lungs, liver, marrow and lymphatic glands.
47 4 days. M. Slight diarrhœa, slight congestion of lungs. *Bact. coli* from blood, lungs, liver and intestinal glands.
50 6 days. M. Diarrhœa, slight congestion of lungs and intestines. *Bact. coli* from blood and all organs.
55 7 days. M. Diarrhœa and constipation, pneumonia with purulent foci. *Bact. coli* from blood and all organs.
57 2 days. M. Diarrhœa, slight congestion of lungs and intestines. *Bact. coli* from lungs and all organs.
58 12 days. M. Inappetence, lobular pneumonia and pleurisy. *Bact. coli* from blood and all organs.
59 11 days. M. Inappetence, slight congestion of lungs. *Bact. coli* from marrow and intestinal glands.
60 9 days. M. Inappetence, no abnormality, *Bact. coli* from blood and all organs.
61 7 days. M. Inappetence, purulent umbilicus, *Bact. coli* from blood and all organs.
62 6 days. M. Diarrhœa and inappetence, purulent umbilicus. *Bact. coli* from blood and all organs.

64 13 days. M. Diarrhœa and inappetence, purulent umbilicus. *Bact. coli* from blood and other organs.

65 13 days. M. Diarrhœa and inappetence, congested umbilicus, pericarditis, *Bact. coli* from blood and all organs.

66 5 days. M. Diarrhœa and inappetence, purulent umbilicus, congested intestines. *Bact. coli* from spleen and intestinal glands.

69 M. Peritonitis, slight congestion of lungs and small intestines. *Bact. coli* from marrow, lungs and intestinal glands.

70 14 days. M. Diarrhœa and dyspnœa, necrotic umbilicus, slight congestion of lungs, necrotic areas in lungs and liver. *Bact. coli* from blood and other organs. *Staph. aureus* (hæmolytic) from lungs.

72 6 days. M. Diarrhœa and inappetence, congested umbilicus and intestines, enlarged glands. *Bact. coli* from intestinal glands.

73 9 days. M. Diarrhœa, inflammation of small intestine. *Bact. coli* (hæmolytic) from lymphatic glands and intestines.

74 3 days. F. Weak, purulent umbilicus, congestion of lungs and intestines, hæmorrhagic lymphatic glands. *Bact. coli* from blood and all organs.

75 9 days. M. Dull, organs normal. *Bact. coli* from blood and all organs.

76 13 days. M. Slight diarrhœa, organs normal. *Bact. coli* from blood and all organs.

77 16 days. M. Diarrhœa, inappetence and dyspnœa, necrotic umbilicus, congested lungs with necrotic foci. *Bact. coli* from blood and all organs.

78 16 days. M. Diarrhœa and inappetence, necrotic umbilicus and congestion of lungs and small intestines. *Bact. coli* from blood, liver and intestinal glands.

79 13 days. M. Dull, necrotic umbilicus, congestion of lungs and intestines. *Bact. coli* from blood and all organs.

80 8 days. M. Diarrhœa and inappetence, necrotic umbilicus, *Bact. coli.* from blood and other organs.

89 7 days. M. Inappetence and diarrhœa, slight congestion of lungs and kidneys. *Bact. coli* from blood and all organs.

90 M. Congestion of lungs and mediastinal glands. *Bact. coli* from blood and all organs.

C. PYOGENES *Infections* (12 *cases*).

F2 7 weeks. Cough, consolidation of lungs, *C. pyogenes* from lungs.

F3 3 weeks. Consolidation of lungs. *C. pyogenes* from lungs.

F6 3 weeks. Hepatization of lungs, multiple abscesses, pleurisy with adhesions. *C. pyogenes* from lungs.

F7 2 weeks. Hepatization of lungs, multiple abscess formation, pleurisy with adhesions, *C. pyogenes* from lungs.

F8 1 week. Consolidation of lungs. *C. pyogenes* from lungs.

F9 4 to 5 weeks. Killed moribund, consolidation of lungs, areas of necrosis and cavity formation, fibrinous pleurisy, *C. pyogenes* from lungs and presternal lymphatic gland.

F11 5 months. Weak, consolidation of lungs, necrotic areas, pleurisy, *C. pyogenes* from lungs and mediastinal lymphatic gland.

7 3 months. M. Pneumonia for a month, areas of lung consolidation with areas of necrosis, pleural adhesions, pericarditis, mediastinal lymphatic glands enlarged, left stifle joint contained blood-stained fluid. *C. pyogenes* from blood, lungs, spleen, liver, marrow, stifle joint, intestinal and mediastinal glands.

8 3 months. F. Pneumonia for a month, hepatization of lungs, pus formation, pleural adhesions, pericarditis, stifle joints contain gelatinous fluid. *C. pyogenes* from blood, lungs, liver and mediastinal glands.
9 1 month. F. Diarrhœa and inappetence, consolidation of lungs, areas of necrosis, enlarged mediastinal glands. *C. pyogenes* from lungs, mediastinal and intestinal glands.
30 9 days. F. Diarrhœa, congestion of intestines, consolidation of lungs, purulent foci, fibrinous pleurisy, *C. pyogenes* from lungs, liver, mediastinal glands.
49 M. Consolidation of lungs, areas of necrosis, pleurisy, *C. pyogenes* from lungs.

Hæmolytic Coccobacillus *Infections* (6 *cases*).

18 5 days. F. Premature, weakly, intestines congested, consolidation of lungs. Hæmolytic coccobacillus from blood, lungs, liver, spleen and intestinal glands.
31 2 months. F. Weak, ulcers on tongue, necrosis in fauces and œsophagus, consolidation of lungs, pleurisy with adhesions, hæmorrhagic mediastinal glands. Hæmolytic coccobacillus from blood, lungs, mediastinal glands.
39 1 month. M. Febrile, cough and coryza, consolidation of lungs with pus in bronchi, hæmorrhagic mediastinal glands. Hæmolytic coccobacillus from lungs and mediastinal glands.
40 3 weeks. F. Weak, dyspnœa, congested lungs, and small intestine. Hæmolytic coccobacillus from lungs.
45 2 to 3 weeks. M. Premature, weak, febrile, coryza, respiratory distress, lungs and trachea deeply congested. Hæmolytic coccobacillus from lungs.
48 2 to 3 months. M. Respiratory distress, coryza, discharge from ear, consolidation of lungs, multiple purulent foci, congested trachea, hæmorrhagic mediastinal glands. Hæmolytic coccobacillus from lungs.

Salmonella *Infections* (2 *cases*).

A2 1 month. Enteritis, general weakness, hæmorrhagic intestinal glands, *S. typhi-murium (Bact. aertrycke)* from spleen, liver, intestinal glands and intestines.
35 1 month. F. Shivering, coryza, congestion of lungs and small intestine, *S. typhi-murium* from intestinal glands and small intestine.

Pasteurella *Infection* (1 *case*).

2 13 days. F. Diarrhœa, congestion and consolidation of lungs, pleural adhesions, blood-stained pleural fluid, *Pasteurella* from lungs and pleural fluid.

Mixed Infections (11 *cases*).

F1 Consolidation of lungs, pus formation. *C. pyogenes* and *Pasteurella* from lungs.
F10 2 to 3 weeks. Consolidation of lungs, *C. pyogenes* and *Pasteurella* from lungs.
20 3 to 4 weeks. F. Premature, weak, discharge from eyes, consolidation of lungs, purulent foci, pleurisy with adhesions. *C. pyogenes* from lungs and spleen. Hæmolytic coccobacillus from lungs.

38 4 to 5 weeks. F. Weak, congested lungs, multiple pinhead purulent foci, pleurisy and pericarditis, *C. pyogenes* from lungs, liver, mediastinal gland and pleural fluid. Hæmolytic coccobacillus from lungs and pleural fluid. *Pasteurella* from pericardial fluid.

51 9 days. M. Diarrhœa, umbilicus necrotic, congestion of lungs, purulent foci, enlarged lymphatic glands. *Pasteurella* from lungs. *Staph. aureus* from lungs.

53 7 days. M. Diarrhœa, weakness, congestion of lungs, small intestines. *Bact. coli* and *Staph. aureus* from lungs.

56 7 days. M. Diarrhœa, congestion of lungs, intestines and umbilicus. *Pasteurella* from blood, liver and mediastinal gland. *Bact. coli* and *Staph. aureus* from lungs.

63 9 days. M. Diarrhœa and inappetence, congestion of lungs, purulent umbilicus, *Bact. coli* and *Ps. pyocyanea* from lungs and intestinal glands.

67 11 days. M. Diarrhœa and inappetence, congestion of lungs, hæmorrhagic mediastinal glands, umbilicus purulent. *Bact. coli* and *Ps. pyocyanea* from lungs.

71 8 days. M. Diarrhœa, dull, purulent umbilicus, *Bact. coli* and *Ps. pyocyanea* from lungs.

85 1 month. F. Abscess formation at side of face, enlarged associated lymphatic glands, some congestion of small intestines. *Bact. coli* from lungs and intestinal glands. Hæmolytic coccobacillus from abscess. *C. pyogenes* from submaxillary gland.

Miscellaneous Group (31 cases).

A1 5 weeks. Enteritis, congestion of lungs and intestines, late lactose-fermenting coliforms from liver and intestines.

F12 6 weeks. Hepatization of lungs, no bacteria isolated.

1 8 weeks. F. Paralysis for 14 days, killed, general lymphatic enlargement, anæmia, no significant bacteria isolated.

4 5 weeks. F. Diarrhœa, some congestion of lungs, enteritis, *Bact. coli* from spleen and marrow. A streptococcus and staphylococcus from marrow.

5 7 weeks. F. Died suddenly, petechiæ over coronary grooves of heart. Hæmolytic streptothrix from lung and bile.

6 5 days. M. Slight diarrhœa, difficulty in walking, killed, some congestion of intestines, synovia of hocks turbid, haemolytic coliforms from intestinal glands.

10 5 days. F. Found moribund, congested lungs, alpha-hæmolytic streptococci from blood, spleen, lungs and liver.

11 2 days. F. Weak, congestion of lungs and small intestines, hæmorrhagic cystitis, blood-stained peritoneal exudate, atypical coliform bacilli from peritoneal fluid, lymphatic glands and joints.

13 3 weeks. F. Pneumonia symptoms, distension of intestines and gall bladder, no significant bacteria isolated.

15 9 days. M. Inappetence, blind, congestion of small intestine, lungs, kidneys and glands. Hæmolytic gelatin-liquefying coliform bacilli (doubtful if *Proteus vulgaris*) from blood, lungs, spleen, pericardial fluid, mediastinal and intestinal glands.

16 3 to 4 weeks. F. Rupture of abomasum with peritonitis.

19 3 weeks. M. Diarrhœa, purulent umbilicus, congested lungs and intestines. *Bact. coli* from intestinal glands and umbilicus.

22 10 days. F. Inappetence, congestion of lungs with purulent foci, fibrinous pleurisy, congestion of intestines. *Bact. alkaligenes* from lungs and liver.

23 5 days. F. Dull, no obvious abnormality. *Proteus vulgaris* from all organs.

27 3 weeks. F. Acute diarrhœa, congestion of right lobes of lungs, hæmolytic anaerobic bacilli from blood, lungs, spleen, liver and mediastinal glands.

28 17 days. F. Found dead, congestion of lungs, hæmorrhagic mediastinal glands. *Bact. coli* from lungs and intestinal glands.

29 4 to 5 months. F. Dyspnoea, blood-stained pleural and peritoneal fluids. Hæmolytic anaerobic bacilli from blood and various organs. (Possibly HCN poisoning.)

33 3 to 4 months. F. Found dead, congested lungs, blood-stained pericardial and peritoneal exudate—probably HCN poisoning.

34 4 to 5 months. F. Partial paralysis of hind limbs, unable to rise, degeneration of muscles of hind quarters, lungs congested, gall bladder distended, no significant bacteria isolated.

36 4 months. F. Died suddenly, blood-stained pleural and peritoneal exudate, few hæmolytic anaerobic bacteria isolated, probably HCN poisoning (from same farm as No. 33).

41 1 month. F. Diarrhœa, no other abnormality, hæmolytic coliform bacilli from intestinal glands.

46 3 months. F. Acute congestion of lungs, intestines and associated lymphatic glands. No significant bacteria isolated.

52 5 weeks. M. Diarrhœa and emaciation, purulent umbilicus, congestion of lungs. *Bact. coli* from liver, bile and intestinal glands.

54 2 weeks. M. Premature birth, weak, some consolidation of lungs, pleurisy, *Proteus vulgaris* from lungs and intestinal glands.

68 11 days. M. Diarrhœa and inappetence, umbilicus contained blood clot, slight congestion of lungs, intestines and intestinal glands. Streptococci from lungs, *Bact. coli* and *Proteus vulgaris* from intestinal glands.

81 12 days. F. Diarrhœa, congested small intestine, enlarged mediastinal and portal glands. Streptococci from lungs, another species of streptococcus from liver.

82 4 days. M. Diarrhœa, congestion of lungs, intestines and associated glands. Alpha-hæmolytic streptococci from lungs, liver, mediastinal glands and bile.

83 2 months. F. Inappetence, congestion of lungs. *Bact. coli* and streptococci from lungs.

84 8 days. M. Weak, inappetence, congestion of intestines, hæmolytic coliform bacilli from intestinal glands.

87 3 months. M. Diarrhœa and signs of pneumonia, choked by piece of bark in œsophagus, commencing pneumonia, atypical coliform bacillus from blood, *Bact. coli* from liver and bile, *Ps. pyocyanea* from lungs and spleen.

88 12 days. F. Diarrhœa, *Bact. coli* from mediastinal gland and liver.

EPITHELIOGENESIS IMPERFECTA NEONATORUM IN A FOAL, POSSIBLY OF A HEREDITARY NATURE.

By H. Berthelsen and K. Eriksson.

From the Department for Pathologic Anatomy (Director, Professor A. Hjärre) and Animal Husbandry (Director, Professor K. Eriksson) at the Veterinary College in Stockholm.

I.—PATHOLOGIC-ANATOMICAL INVESTIGATION.

By H. Berthelsen.

Introduction and History.

It is difficult to achieve a satisfactorily detailed and systematic classification of malformations of the skin (Bettmann,[1] Cockayne,[3a] Darier,[5] Gans,[8] Heller[32]). Gans[8] classifies them into hypo-, hyper- and dysplasias, cysts and nævi. In so far as the first three are concerned, he further mentions general ones and circumscribed ones. In the present article only the hypoplastic malformations are of interest. We shall therefore ignore and leave out of account the others in what follows.

The majority of hypoplastic malformations have been described in man, and mostly in children, while informations in respect of animals is scanty. Those which we encounter in the literature on the subject seem to fall into two large groups from a morphological point of view:—

(1) Defects in the integumentum commune.

(2) Hypoplasias of the integumentum commune and appendices (sebaceous and sweat-glands, as well as hair).

Amongst the defects coming under group (1), those which appear to be most frequent are the minor ones on the vertex of newly born infants (Burger,[2] Hoffmann,[11] Keller,[16] Neumann,[23] Oing, [24] Walz[31] and several others). As to their etiology, different opinions have been advanced, *e.g.*, violence (Hoffmann[11]), intrauterine inflammation (Oing,[24] syphilis), fusion with amnion (Keller,[16] Neumann,[23] Oing,[24] etc.), obstructionary malformation (Walz[31]), etc. More infrequent are integumentary defects on the rest of the body. Such are described *inter alia* by Lundwall[20] (good history), Heinrichsbauer[10] and Köhler.[18].

Cases belonging to group (2) are described by Tendlau,[30] Loewy and Wechselmann,[19] Jones and Atkins (cit. Bettmann[1]). In respect of Tendlau's[30] and Loewy-Wechselmann's[19] very interesting cases (observations and experiments *intra vitam*) there exists a well-founded suspicion of hereditary disease. Otherwise it is striking how scanty are as a rule the genetic items of information.

I have only succeeded in meeting with a few cases in animals, all of them probably belonging to group (1).

Siedamgrotsky's[28] case concerned a newly-born, full-grown calf. No genetic information. The calf displayed great deviations from normality,

in that it had developed a frog-like lump of flesh with peromelia, kyphose, scoliosis, defective eyes and ears, and so on. " Weitaus am interessantesten sind die Verhältnisse der Haut. Der verkrüppelte cadaver ist nämlich zum grössten Theile von einer Bindegewebslage überzogen, welche weder eine Epidermisdecke noch Haare zeigt, und durch die Fett, Muskeln, Knochen etc., hindurchschimmern. Die normale Epitheldecke und Papillen tragende Schleimhaut endet ziemlich scharf abgesetzt am Umkreise der Maulnasenöffnung. Freiliegende normale Haut mit Rete Malpighii Epidermis, Haaren und Drüsen findet sich nur auf dem Rücken über die Lendenwirbeln und den Kreutzbeine in Form einer unregelmässig ovalen, schräg zur Längsrichtung des Embryos liegende Platte von 17, resp. 12 ctm Durchmesser, welche mit einem schmalen Fortsatze nach hinten den After umfasst. Die Ränder dieser Hauptplatte sind scharf abgesetzt, indem plötzlich die Epidermisdecke und die Haaren fehlen, und das Corium sich in den dünneren Bindegewebsüberzug des Körpers fortsetzt." In this plate there were some small holes, and from these there issued a system of epidermis channels which sent ramifications to all the rudimentary extremities. Isolated, minor epidermis channels existed also on both the rudimentary ears. The walls of the channels contained all the components of the skin, and was inside clothed with hair. The diameter of the channels varied between 0·4 and 2·5 cm. Here and there they formed some blind alleys with a diameter of up to 10 cm. Siedamgrotsky[28] considered that the cause of malformation might be due to a cleavage formation in the ectoderm during the tadpole stage, so that the skin on account of contraction had been rolled up.

Stoss's[29] case related to a foal's head. The lower jaw was bent ventrally about 130°, obviously on account of fœtal violence. Furthermore, there occurred some minor skin defects on eyelids, vertex, forehead, ridge of nose, and upper and lower lip. The normal skin terminated abruptly. The skinless spots were covered with a cicatrised tissue which did not contain any papillæ, hair or sweat glands.

Hadley[9] described in the calf several cases of a probably hereditary defect of epithelium on the lower parts of the extremities. The disease was confined to a definite locality in North America and to a definite breed. Hadley does not give any pathologic-anatomic description of his cases. He suggests the name *epitheliogenesis imperfecta neonatorum.*

Joest[12] mentioned cursorily that an otherwise greatly malformed calf foetus lacked skin on the rudiment of the left hindleg. The same fœtus also showed in the neighbourhood of all the rudimentary extremities a subcutaneous ramified system of channels which on their interior were clothed with hair.

Grundt[33] described two foals without any skin on the distal parts of the extremities. He did not interpret these as a lack of skin but rather as an intrauterine inflammation, with a discharge of epidermis. He also mentions the occurrence of dermoid cysts in the portions without skin.

As has already been mentioned, a satisfactory classification of diseases or defects coming under this heading is difficult to draw up. A causal one carried out is at present scarcely possible. A formal one also presents some difficulties. It seems to me, therefore, that we might for the time being in the first instance group them along certain great causal lines, *e.g.*, one group of cases of fœtal violence, another for those of an amniogenous

nature, a third for distinctly endogenous ones, a fourth for cryptogenous ones, and so on. Within each main group we might then for further classification apply a formal principle.

Of course, it is feasible that later on, especially in so far as the animals are concerned, we shall get farther in the differentiation between heritable and non-heritable ones than is now the case (Cockayne[3, 1]).

Nomenclature.

As to the nomenclature, we see in respect of human beings in cases of defects simply the term "skin defect." Bettmann[1] seems to lump together all the said defects under the term *atrophia cutis congenita*. Cockayne[3A] uses the term "*aplasia cutis congenita.*" In regard to domestic animals Kitt[17] has on the basis of Siedamgrotsky's[28] and Stoss's[29] cases suggested the term *perodermia* (defective development of the skin). Hadley[9] uses, as mentioned above, the term *epitheliogenesis imperfecta neonatorum*. I do not at present wish to discuss the nomenclature for the cases already described, but I think that for cases of the nature which I am going to describe hereafter Hadley's term fits very well. I therefore suggest that the term "*Epitheliogenesis imperfecta neonatorum*" be used for cases of innate aplasia of the epidermis with retained corium.

In what follows a description will be given of such a case in a foal.

Case.

This relates to a whole carcase of a newly-born brown foal (Fig. 1) sent to the Institute (0·258/34). It appears to be fully developed, in that I_1 and I_2 as well as P_1—P_3 have broken out in all jaws. It looks as if it had lived after birth and been killed by a cut in the throat. On external examination the foal appears of normal size. Skin and hair on the head, body and tail, and the proximal parts of the extremities show no macroscopic abnormities. On the other hand, the distal parts of the extremities are seen from a little above the carpus and tarsus to be almost completely deprived of epidermis and hair. Instead of this these parts are mostly covered with an approximately 1 mm. thick, reddish, moist film, through which it is possible to see the underlying parts of tissue (Fig. 1). The bulk of this film is quite smooth and has a uniform appearance. It is only around the tarsus that one can see in it here and there greyish-white, rather firm nodules, varying in size from a mere point to a pin-head. And also on the dorsal side of the third phalanx of the two hind legs and on the right fore leg a couple of small nodes are present *below* it. The last-mentioned nodes vary in size from a hempseed up to a pea, and have a firm consistency and a whitish-grey, dry cut-surface. They can be moved in relation to the basis, but are fairly well attached to the film. In the film can also be seen minute folds around the joints.

Furthermore, we encounter in the *border areas* between the film and the normal skin some changes which will be described rather more in detail. The hair terminates everywhere abruptly with a somewhat wavy border line slightly proximal to the carpus and tarsus (Fig. 1). The normal epidermis terminates simultaneously with the hair with a slight wall. In a distal direction there begins now (1) the thin film directly, or (2) the thin film with

the minute white nodes, or (3) the thin film covered by a thin abnormal layer of epidermis. The areas mentioned under (2) and (3) extend from 1 to 10 cm. in a distal direction reckoned from the hair border. Farthest down they extend on the dorsal side of the hock. The areas mentioned under (1) and (2) have already been described in the preceding. As regards (3), it displays a somewhat varying appearance. In some places it is almost smooth and brownish-grey in colour, but in the majority of places it is more or less granular, being plentifully spotted with thickened epithelium varying from a pin-head up to half the size of a confetti (hyperkeratosis). Here and there it contains also a few minute hairs, mostly in and around the granular portions. The last-mentioned thin layer of epidermis therefore

Fig. 1.—Foal with extremities devoid of epidermis. On the dorsal side of the hock is seen a small part of the granular film.

occurs as a kind of continuation of the normal epidermis. Furthermore, we encounter in the border areas some minute isolated spots of the same kind lying strewn about on the thin film.

It thus seems as if in some areas there is a direct transition between the area covered by normal epidermis and the one where the epidermis is quite aplastic thin film, while in others we have the transitions from a normal epidermis to granular film, then to thin film with minute nodules in them, and finally thin film.

The autopsy did not show any other notable changes, but it may be mentioned that the lungs were aeriferous and the foramen ovale and the ductus Botalli open.

Photos and microphotos taken by H. Berthelsen.

Microscopic Examination.

The *macroscopically normal epidermis* on the proximal parts of the extremities does not show any microscopic changes (Fig. 4).

The thin film (Figs. 2 and 5) consists of a condensed corium over a subcutis which is plentifully vascular but otherwise apparently normal. There is no sign of epithelium on the corium, and in the film there are neither hairs, hair muscles, sebaceous or sweat glands, nor any signs of such. No *stratum papillare* occurs. The film might therefore very well be interpreted as a condensed *stratum reticulare*. It is built up of tightly packed, coarse fibrillæ of connective tissue which in van Gieson-stained sections assume an intensely red colour. The connective tissue fibrillæ run on the whole parallel with the surface and have a straighter course than is the case in normal corium (Figs. 4 and 5). In the superficial parts the fibrillæ lie very close together and the nuclear quantity is small here, and in some spots there is none at all. In the deeper parts the fibrillæ lie more thinly and the

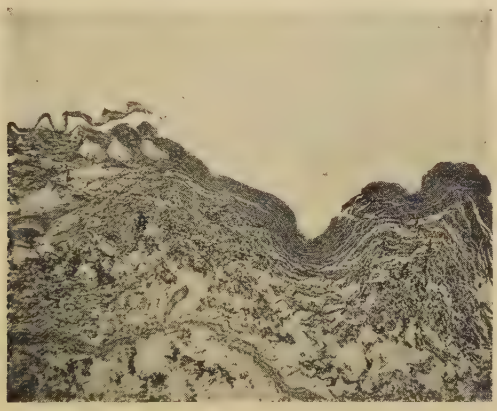

Fig. 2.—Border area between normal skin (on extreme left) and the thin film. Under the epidermis are seen some sebaceous glands. On the film on the extreme right is seen the plicated formation mentioned in the text. Low magnification.

nuclear quantity is greater. The appearance of the nuclei is that of normal dense connective tissue. In some spots the corium is stained up to the surface with the same intensely red colour, but in some spots one can see that the outermost layers change slightly into yellow, without showing any more conspicuous differences from entirely red-coloured ones. The yellow tint is also the same as that of the horny layer in the normal epidermis. The yellow tint can *inter alia* be met with in the dorsal walls of the third phalanx. There are even transitions between red- and yellow-stained fibrillæ. In silver-impregnated sections (Foot and Foot[6] modification) the strongly red-stained portions in van Gieson sections assume a dark red tint, while those with a yellow tint are found to consist of a dense layer of argentophile, fairly slender fibrillæ. This is especially prominent on the dorsal side of the third phalanx. Here the layer has a thickness of something like 50μ, while elsewhere in the thin film it may be about 10μ. *Elastic fibrillæ* (Weigert's resorcin-fuchsin) occur plentifully in the deeper layers of corium, while the more superficial dense layers contain only very few. The elastic fibrillæ run in waves, but on the whole parallel with the surface.

In the deeper layers of corium and in the subcutis there are plenty of

minute vessels filled with blood. In and immediately below the argentophile layer on the third phalanx these vessels are lying very close together. They give the impression of being the beginning of a subungular net of veins.

Border Areas.—Ad. (1). All the layers of the normal epidermis terminate abruptly with a slight swelling (Fig. 2 and 3). The epidermoid formations, hair, sebaceous and sweat glands also terminate at the same time. In a distal direction the body cover now consists of corium, already described. In some places it looks as if the film were showing an indication of the formation of a papillary body. The *elastic fibrillæ* are layered in the thin film more thinly than in the normal skin, while the total number does not appear to be altered to any great degree. The outermost layers of the film are free from elastin.

Ad. (2.) On the whole like 1. The small nodules consist of rudimentary tendencies to sebaceous glands in the corium. Here and there one can see hair withour hair-sheaths. *Elastic fibrillæ* occur rather plentifully, mixed with collagen. The course and distribution is the same as in the thin film.

Ad. (3.) These portions show a variegated picture (Fig. 4). The normal

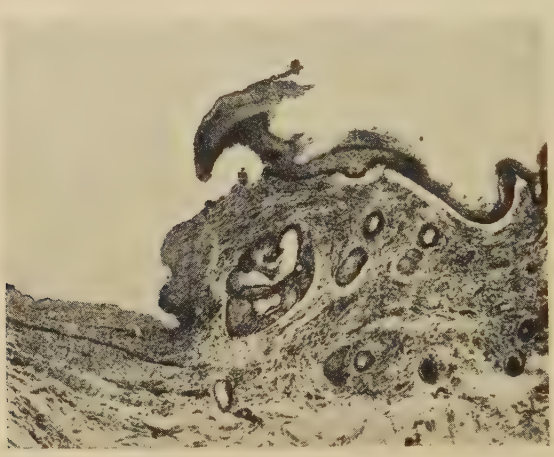

Fig. 3.—Border area between normal skin (on right) and thin film. The epidermis terminates abruptly with a protuberance. The epidermoidal appendages terminate simultaneously. Medium magnification.

epidermis terminates abruptly, as described in the preceding. There then begins an area with sometimes aplastic and sometimes hyperplastic epidermis. The former areas show pictures similar to those in the thin film, and the latter a hyperkeratotic appearance. Hair and sebaceous glands occur irregularly. Sweat glands are lacking. *Elastic fibrillæ* occur rather plentifully.

Between the areas here mentioned one can observe transitions.

One of *the nodes on the third phalanx* which was microscopically examined has an ovoid cut-surface with the poles respectively distal and proximal. It lies on the border between the corium and subcutis and is covered with a thin layer of corium. The node consists mainly of keratinised epithelium cells (stand out beautifully in elastine stain). Along the edge turned towards the depth there lies a layer of polygonal epithelium cells (3 to 4 in width). The nuclei are oval or more rounded, with a sparse chromatic net. The cells remind one of those in the outer layers of a hair, and they pass fairly evenly over into the keratinised ones. On the outside of this vascular layer (*i.e.*, lower down) there is a still narrower layer of closely packed cells

with elongated dark nuclei. These are on one side not sharply delimited towards the polygonal ones, and on the other side not sharply from the surrounding connective tissue cells. The last-mentioned layer of cells extends some distance past the poles but is gradually flattened into nothing. Along the edge facing the surface there lie the keratinised cells very close to the corium cells. In serial sections on one half of the node no connection could be traced between the node and any epithelial cells that might possibly lie on the surface. The *elastic fibrillæ* show on the whole the same conditions as in the thin film. No elastic fibrillæ can be demonstrated *in* the nodes.

The pathological changes in the case described I interpret as an endogenously conditioned, imperfect epitheliogenesis localised to the extremities. Whether this is hereditary or not is difficult to decide. In this respect I beg to refer to the following account by

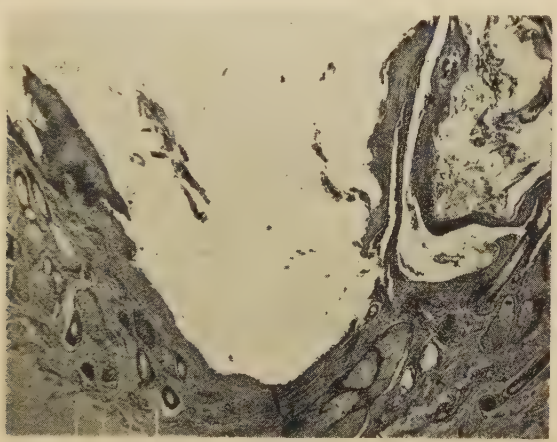

Fig. 4.—Border area between normal skin (on left) and granular film. The epidermis terminates on the left abruptly with a protuberance. Then follows an area with entirely aplastic epidermis. On the right hyperkeratosis. The distribution of hair and sebaceous glands is seen from the figure. Medium magnification.

Professor Eriksson. The uniform spread of the defects and the total lack of inflammatory changes exclude amniogenesis and inflammatory genesis, as well as fœtal violence.

Of previously described cases, it shows the greatest macroscopic agreement with Hadley's.[9] Whether this agreement also extends to the histological picture I am unable to decide on account of Hadley's rather meagre information on this point. My case shows fairly good macroscopic and partial microscopic agreement with Heinrichsbauer's,[10] apart from localisation. Nevertheless, he found a defective epidermis, but feebly developed epidermoidal secondary organs. The possibility of heredity is also a parallel. Siedamgrotsky's[28] case seems also capable of being included in the same group.

As regards the nodes on the wall of the hoofs, I am very strongly inclined to interpret them as heterotopic epidermis formations, possibly abnormal hair rudiments. According to Grundt's[33]

description he also seems to have observed similar formations, which he regards as dermoid-cysts.

The relation of the corium to van Gieson and silver impregnation seems to indicate that the bulk of the corium consists of collagenous connective tissue, while some superficial portions consist of reticulum. This is on the assumption that, as is often the case, by collagenous fibrillæ are meant such as are stained red by picrofuchsin, and by reticular ones such as turn black on being impregnated with double salts of ammonia and silver (Foot[7]). Opinions on the specificity of these reactions are, however, somewhat doubtful. Foot,[7] Maximow,[21] Nageotte and Guyon,[22] Orsos,[25]

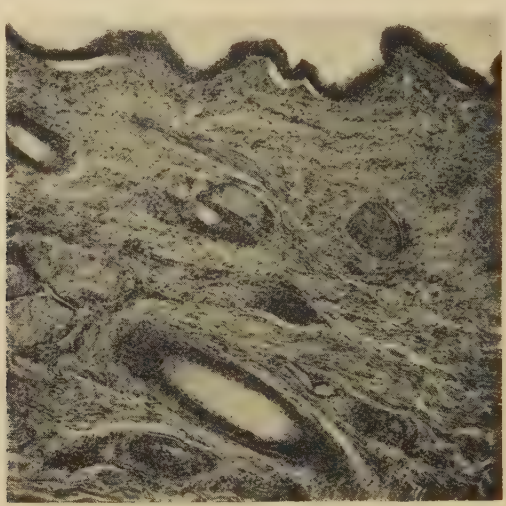

Fig. 5.—Normal skin from proximal portion of one extremity. At the top epidermis, in the centre corium, at the bottom subcutis, with hair, sebaceous and sweat glands, and musculi pilorum. Medium magnification.

Rinehart[26] and others consider that no sharp delimitation exists, either histio-morphologically or chemically, between these two substances. The different reaction to van Gieson and silver may be due to a different fineness and stratification of the fibrillæ. Maximow[21] has thus in tissue culture demonstrated that collagenous fibrillæ only consist of densely stratified reticular ones. Foot states (in a discussion with Callender[2]) ". . . we are dealing with two substances closely related to one another. In the case of reticulum, collagen may be associated with some other substance that gives this tissue its characteristic argyrophilic tendencies—the utility and resources of staining methods are exhausted in this connection. We cannot explain the difference between reticulum and collagen by means of staining methods alone. We must also approach the question from a chemical standpoint." It would here take us too far to enter upon the different opinions regarding

the chemical difference between the two substances. We refer in this matter to Foot.[7]

The result of the histio-chemical reaction in our case therefore only tells us that the corium seems to have a different structure in the places with the different reaction. As regards the film above the third phalanx, it is quite natural to consider the superficial argentophile tissue in relation to the great number of vessels as a hint of the formation of subungular tissue.

Fig. 6.—Thin film with one plication. The density of the layering of the connective tissue is clearly seen, and also its parallelism with surface. Medium magnification.

II.—GENETIC INVESTIGATION.

By K. Eriksson.

In order to judge the question whether the case of *epitheliogenesis imperfecta neonatorum* in a foal described in the preceding pages by Harald Berthelsen can be of an hereditable nature, the following information has been obtained from the owner of the deformed foal's dam, and from Mr. O. Svensson, District Veterinary Surgeon, of Mörbylånga.

The dam, Stjärna, was born in 1925 on the Island of Oland, and is said to have given birth to three foals, one by the Ardennes stallion Mumrik 2566, and two by the Ardennes stallion Gambrinus 1664. The first-mentioned foal is stated to have been normal, while the two last-mentioned, one of them being the foal here concerned, had been deformed. In both cases it was a matter of defects of the epidermis. In the foal which has not been the subject of pathologic-anatomical investigation the epidermis defects are stated to have affected three legs and the entire croup.

Both the mare Stjärna and the stallion Gambrinus are said to be free from both skin and hair defects, nor is it known that the stallion Gambrinus has produced deformed foals by other mares.

The pedigree of the deformed foal, as far as it could be ascertained, is seen from the following :—

FILLY WITH *Epitheliogenesis imperfecta neonatorum*, BORN ON MAY 24TH, 1934.

Gambrinus 1664	Jonas 42662 B.	Louis Brun 10162 B.	Moka 3176 B.	Mouton
				Louise
			Toinette	
		Cécile 43963 B.	Bric-Broc 7624 B.	Jupiter
				Mélie
			Tolie 987 B.	
	Simonne 9395 B.	Cognac de Bierwart 23484 B.	Robert II 4202 B.	Hector
				Sarah
			Brune 6393 B.	Alexis
				Bara I
		Camail 9395 B.	Lancier I 4188 B	
			Mouchette 6387 B.	Jean I
				Sarah
Stjärna	Major de Leuze 1150	Farceur d'Abée 35486 B.	Bayard d'Abée 16682 B.	**Masillon 6778 B.**
				Baie d'Abée 12895 B.
			Alice d'Abée 40529 B.	**Masillon 6778 B.**
				Pauline d'Abée 12893 B.
		Charmante de Leuze 52253 B.		
		Half Ardennes stallion		

It will be seen from this that the pedigree of the mare Stjärna is not known on the mother's side. The maternal sire is said to be a half-Ardennes stallion. The pedigree also shows that Stjärna's grandsire, Farceur d'Abée 35486 B, is the product of half-brother and sister breeding, and that Gambrinus' dam, Simonne 9395 B, in the third ascendant generation has the mare Sarah for her grandsire's dam and grand-dam's dam. As far as their pedigrees are known, no relationship exists between Stjärna and Gambrinus. Nevertheless, it is quite possible that joint ascendants may occur in earlier ascendant generations, though this is not shown in the pedigree.

It is not possible to draw any safe conclusions from the available data as to the hereditable nature of the malformation. To do this, the material is far too scanty and incomplete. If any heredity were to form the basis of the malformation or deformity, it must obviously concern one (or more) recessive factors of heredity, inasmuch as the sire and dam of the foal as well as their ascendants, as far as they are known, have been normal. There should then exist a recessive offshoot in analogy with the segregation of lethal and sublethal factors, which are known in quite a large number of domestic animals. The offshoot of red-haired calves from black-freckled ancestors also supplies an example of genetic analogy. The present case reminds one chiefly of the cases of *Epitheliogenesis imperfecta neonatorum* described by Hadley. But his calves, apart from epithelial defects in the extremities, also showed defects of the mucous membrane of the buccal cavity and malformation of ears and hoofs. In the literature on heredity there are also to be found numerous cases of malformations of epidermis and other kinds, both in animals and human beings, due to segregation of recessive defect-factors in the homozygotic form.

It is quite feasible to assume that the *epitheliogenesis imperfecta* described might be due to a similar recessive defect-factor. The sire and dam of the foal would then both have been heterozygotic for this tendency. The fact that two half-brothers and sisters have been born with similar defects speaks in favour of the probability of this assumption.

On the other hand there is in the pedigree no demonstrable joint ascendant for the sire and dam in the nearest generations, which might be imagined as having transmitted the recessive tendency to the sire and dam. But there is nothing to prevent a recessive tendency existing within two not closely related lines.

In monohybridous segregation we would furthermore have expected three normal foals to one deformed after pairing between Gambrinus and Stjärna. The observed cases of merely two foals which were both deformed shows a deviation from the theoretical expectation, which nevertheless, in consideration of the paucity of numbers, comes under the heading of statistical defective limits.

The mean error in the theoretically expected monohybridous segregation for two is $\sqrt{\frac{1{\cdot}5 \times 0{\cdot}5}{2}}$ and the difference between the data observed and those expected in each two is 1·5, *i.e.*, $\frac{\text{diff.}}{\text{m}} = 2{\cdot}45$.

It is thus possible that we have here to deal with homozygotic segregation of a recessively hereditable tendency. To supply positive evidence and proof of the correctness of this assumption would be possible only by carrying out some breeding tests on a larger scale, which for quite obvious reasons is out of the question.

Since *epitheliogenesis imperfecta* in a foal has so far been observed only in the case here described, we may assume that the supposed hereditary factor very seldom exists. The circumstance that two, in so far as is known unrelated, individuals can be assumed to be the vectors of the tendency, points, however, to the possibility that in future similar cases may turn up. This might in particular be expected in in-breeding between the descendants of the stallion Gambrinus.

Summary.

The authors describe a case of circumscribed aplasia of epidermis (termed *epitheliogenesis imperfecta neonatorum* Hadley), possibly of a hereditary nature. A detailed macro- and microscopic research has been made and genetic conditions have been examined as far as circumstances permitted.

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A NOTE ON ACQUIRED IMMUNITY TO *THEILERIA ANNULATA*.

By M. Sturman.

Veterinary Surgeon, Haklait Cattle Insurance Company, Haifa, Palestine.

The following observations were made in a limited area in Emek Jezreel comprising seven villages and settlements. The conditions for the observations were ideal for the following reasons :—

(1) The district is heavily infested with *Theileria annulata*.

(2) The number of cattle is 1,450 *circ.** and the piroplasmosis history of every individual is recorded. In every case the diagnosis is established by blood examination made either by the Government Laboratory, Department of Parasitology of the Hebrew University, or the author. It is therefore easy in all cases to determine whether we are dealing with a first attack or a new infection (or relapse).

Theileriasis is present throughout the whole year but the majority of cases occur between May and October. The following table shows the monthly distribution of 273 cases of piroplasmosis, of which 258 were Theileria, in 1933 :—

March	... ...	4	September	... ...	51
April	... ...	6	October	... ...	25
May	... ...	39	November	... ...	17
June	... ...	33	December	... ...	14
July	... ...	24	January	... ...	9
August	... ...	44	February	... ...	7

The distribution varies in different years according to climatic variations. In some years ticks appear earlier and the number of cases in April is correspondingly larger.

Restricting ourselves to the years 1933 and 1934, the following results are obtained.

	Total No. of Cattle.	*Total No. of Cases.*	*Total No. of Deaths.*
1933	1,450	258	46 = 17·8 per cent.
1934†	1,450 *circ.*	104	16 = 17·4 per cent.

* The following breeds occur—Arab-Holland, Damascus-Holland, Beirut-Holland and Damascus. There are practically no pure native cattle.

† The figures for 1934 are up to the end of September, up to which date the author's personal experience is limited.

Analysing these figures further, the following results are obtained :—

Year.	*Total No. of Cases.*	*Previous history of* Theileria annulata *within 3 Years.*	*Deaths.*	*First Attacks.*	*Deaths.*
1933	258	114	0	144	46 = 32 per cent.
1934	104	60	0	44	16 = 36·3 per cent.

An examination of the above findings leads to the following conclusions :—

A previous attack of *Theileria annulata* does not confer immunity against further attacks within a period of three years, but the mortality from such attacks is nil, as compared to a mortality of about 35 per cent. in first attacks.

Not only is the mortality reduced to nil as a result of previous attacks, but the clinical course is mild (a temperature up to 41° C. from one to four days) and, what is most important for the farmer, the loss of milk is very slight.

The total loss in milk as a result of second attacks is from 25 to 100 litres per cow, whereas in animals recovering from first attacks the loss of milk is as much as 5,000 litres in a severe case and an average of no less than 1,000 litres per milch cow. Another important fact is that there were no cases of abortion among the infected cases with a previous history of *Theileria annulata*, whereas abortion is common in first attacks.

Between 1933 and 1935, 205 calves in this district were inoculated with *Theileria annulata*; of these one had an attack two years, and two one year, after inoculation, and all three recovered.

It is impossible to be certain whether the attacks in animals with a previous history of Theileriasis are relapses or are new infections, but the fact that the majority of cases occur in the tick season and very few in the winter months suggests that they are mainly true reinfection.

Summary and Conclusions.

A total of 362 cases of *Theileria annulata*, of which 62 were fatal, was observed in 1933 and 1934.

One hundred and seventy-four cases had a previous history of *Theileria annulata* within the previous three years and none died. One hundred and eighty-eight cases were first attacks and 62 died.

The course of infection in animals with a previous history of *Theileria annulata* was mild, and the loss of milk was from 25 to 100 litres per cow as compared to no less than 1,000 litres per cow in animals recovering from first attacks. An animal recovering from *Theileria annulata* may be reinfected within three years after the recovery but the reinfection causes no mortality.

STUDIES ON BOVINE MASTITIS.

XI.—FURTHER OBSERVATIONS ON THE CONTROL OF CHRONIC STREPTOCOCCUS MASTITIS.

By A. W. Stableforth, S. J. Edwards and F. C. Minett,

Research Institute in Animal Pathology, Royal Veterinary College, London.

In 1933 we gave an account of a herd from which chronic streptococcus mastitis due to *Str. agalactiæ* had been eradicated by the segregation and eventual sale of infected cows. This confirmed the generally accepted belief that this disease is strictly contagious and that, therefore, it should be possible to control it by the simple procedure of avoiding direct transmission during milking. At that time similar control measures were also being carried out in six other herds, *viz.*, Herds A, C, I, J, K and L and the purpose of this report is to detail the results of this work.

It is evident from the literature on the subject that laboratories concerned with mastitis are now giving more attention to control of the disease by segregation, with or without treatment of infected cows. Vaccines, autogenous or other, have gradually fallen into disrepute because, although some authors believe they reduce the more obvious manifestations of the disease, they do little or nothing to diminish the number of infected udders or to prevent the spread of the disease. In accordance with this view, many workers who have tried vaccination, *e.g.*, Seelemann, Plastridge, Anderson, White and Rettger, have replaced it by segregation methods, whilst in Germany a voluntary state-assisted scheme of control based on segregation of infected cows and their treatment with " entozon " was initiated in April, 1934.

Among the more recent efforts at vaccination or eradication reference may be made to the following.

Seddon and Rose (1934) employed killed vaccines in a herd of about 100 cows over a period of five years. No controls were kept. The amount of obvious mastitis was reduced both in respect of numbers of cases and severity, but the incidence of streptococcus infection was unaltered. Similar findings were reported by Krage and Gipmann in 1931.

Plastridge, Anderson, White and Rettger (1934), working in five herds containing from 25 to 50 cows each, concluded that heat-killed vaccines did not adequately control streptococcus mastitis, while, on the other hand, hygienic measures and segregation of infected cows were found to be effective. From one herd the disease was said to have been eradicated and full details are promised later.

In 1933 we referred to attempts by other workers to control mastitis by segregation. Thus, Seelemann (1932) described the promising results obtained in two herds in which, purely by segregation of infected cows,

infection was reduced respectively from 40 to about 10 per cent. in fifteen months, and from 70 to 20 per cent. in two years. In the latter herd it was reported (1933) that by combining entozon treatment with segregation the infection had been reduced to 4·2 per cent. Seelemann and Hadenfeldt (1933) reported on eleven other herds in which control of the disease had been attempted by segregation of cows found to be infected by three-monthly cultural examination of the milk, with or without subsequent treatment of infected cows with entozon. Their results are best given in the form of a table.

Herd.	*No. of Cows.*	*Original Incidence (per cent.).*	*Final Incidence (per cent.).*	*Period.*	*Control attempted by*
S	177	42	29	2 years	Segregation alone
Ko	180	54	23	4 ,,	
D	65	60	47	2 ,,	
J	50	20	10	2 ,,	
L	138	43	30	3 ,,	
R	134	70	8·6	4 ,,	Segregation and "entozon" treatment
Do	88	42	5·4	2 ,,	
H	81	52	10	2 ,,	
G	80	13	5	2 ,,	
F	22	100	0	5 ,,	
Q	200	45	7	2 ,,	

In all herds new infections continued to appear. It is seen that where entozon was used infection was considerably reduced and in one herd (F) was eradicated, whereas in herds in which segregation alone was adopted eradication was not achieved in any case and the incidence was only moderately reduced.

According to yet other reports from Germany, eradication or a striking reduction in the proportion of infected cows may be brought about by a combination of hygienic precautions in milking, with segregation and treatment of infected cows with entozon. The reports, however, do not show how often new infections arose, and, therefore, to what extent the reduction in the number of infected animals was due to continued treatment of old and newly infected cows or to the other measures used. The literature regarding the efficacy of entozon treatment will be dealt with in a subsequent report.

Methods Used for the Examination of Milk Samples.

The methods used were similar to those given by Minett, Stableforth and Edwards (1933), to which reference should be made for details. They consisted essentially of: (1) The use of fore milk, after rejecting one or two streams. Samples were taken from each quarter direct into specially-made centrifuge tubes of 10 c.c. capacity. (2) Observation of the chemical reaction, and of the amount and character of the deposit after centrifuging. (3) Plating of the deposit in blood agar plates if it was 0·01 c.c. or above [since 1933, blood agar containing crystal violet 1 : 500,000 and æsculin 0·1 per cent. (Edwards, 1933)]; sowing of deposit into an enrichment medium consisting of serum glucose

broth containing 1 : 200,000 crystal violet (Edwards, 1930) if it was below 0·01 c.c. (4) Verification of streptococcus colonies by the fermentation and other tests previously outlined. Since 1932 growths have also been checked serologically, using sera specific to the types already defined (Stableforth, 1932).

Notes.—The term " Infected " in this report signifies cows infected with *Str. agalactiæ*. The terms " Group II " and " Group III " refer to mastitis streptococci so designated in previous studies (Minett, Stableforth and Edwards, 1929; Edwards, 1932).

Herd A.

In 1929, when the work was started, the milking herd numbered 55 animals, *viz.*, 45 Shorthorns and 10 Ayrshires. During 1930 most of the Shorthorns were replaced by Ayrshire heifers, the numbers of which have been gradually increased year by year until the end of 1935 the herd consisted of 146 animals, nearly all Ayrshires. Latterly, the herd has been entirely self-contained. It is free from tuberculosis, certified milk being produced, and the proportion of cows reacting to the test for contagious abortion is now negligible. Milking is carried out by machine thrice daily. Hand stripping is not done.

The usual practice has been to retain in the herd animals infected with *Str. agalactiæ* and to segregate them at one end of the milking shed or to put them into a special " infected " shed or line. The arrangements have varied from time to time according to the number of infected cows and the accommodation, but infected animals have invariably been milked last or with a special machine unit. The owner has also taken immediate steps to prevent spread of disease from any animal showing signs of udder disturbance.

Herd A.

Year.	*State of Herd at end of Year.*			*Additions, Removals and Cows which became Infected during previous 12 Months.*				
	Total.	*Healthy.*	*Infected.*	*Healthy became Infected.*	*Healthy Added.*	*Healthy Removed.*	*Infected Added.*	*Infected Removed.*
1929	55	50	5 (9·1)	—	—	—		—
1930	71	68	3 (4·2)	1 (2·0)	71	52	0	3
1931	112	103	9 (8·0)	6 (8·8)	48	7	0	0
1932	126	78	48 (38·1)	50 (48·5)	37	12	1	12
1933	128	84	44 (34·5)	13 (16·7)	40	21	2	19
1934	159	113	46 (28·9)	12 (14·3)	53	12	0	10
1935	146	117	29 (19·9)	6 (5·3)	38	28	0	23

" Healthy " and " Infected " refer to cows free from or infected with *Str. agalactiae*. " Healthy became Infected " refers to animals which became infected during the year stated, but which were in the healthy section on December 31st of the previous year; the percentage is calculated on the number in the healthy section at that date. "Healthy Added " and " Infected Added " refer to animals which were added to the

herd during the year in question and which were free from or infected with *Str. agalactiae* respectively at their first test. "Healthy Removed" and "Infected Removed" refer to healthy or infected animals (whether infected at first test or subsequently) which died or were sold during the year in question.

The figures in brackets express percentages.

Of the 29 cows classed as infected in 1935, 9 had been free from infection for at least two years. The number of cows actually infected at the end of 1935 is, therefore, 20, and the real incidence 13·1 per cent. (See comment.)

Since 1929 milk samples have been examined at three-monthly intervals from all animals in milk, including those in the infected section. Additional samples have been taken seven to ten days after calving, and from any animal which showed abnormality of the udder or milk. It is seen from the table relating to Herd A that the number of cows at December, 1929, was 55 and that 290 ("Healthy added" *plus* "Infected added") have been added since. Of these 345 cows, 138 were under observation for one lactation or less, 76 for two, 66 for three, 32 for four, 24 for five and 9 for six lactations. The number of tests made on the milk of each cow was from 2 to 22, a total of 2,482 tests, or an average of a little over 7 for each cow.

Comment.

This herd is notable in the first place because, although the original incidence of *Str. agalactiæ* was below 10 per cent., milk samples were regularly examined and infected cows milked last or partially segregated, the number of new infections with *Str. agalactiæ* showed a marked increase in 1932, nearly half of the cows in the herd showing infection in that year. The number of new infections has since gradually decreased, 16·7 per cent. being found in 1933, 14·3 per cent. in 1934, and 5·3 per cent. in 1935. At the time of this report the total number of new infections which have appeared among the 337 cows passed into the healthy section of the herd is 88. More than half of the infected cows were retained for a long time, chiefly on account of the high incidence of other forms of mastitis in this herd, with the result that at December, 1934, the section of the herd described as infected still formed 28·9 per cent. of the whole, whilst at November, 1935, it formed 19·9 per cent. A striking feature of these new infections with *Str. agalactiæ*, however, is that in 29 cases the organisms have apparently disappeared from the udder. In nearly all of these cases the streptococci were detected once only and usually only by means of the glucose broth enrichment medium, in spite of numerous examinations during the following one to three years. Such cows have, however, always been kept in the infected section and the number shown as infected is, therefore, unduly high for all years since 1932. The number of cows actually infected at December 31st, 1934, was 19 per cent., whilst at November, 1935, it was 13·1 per cent. In those infections which became permanent the degree of disturbance was slight at first, but during the last

twelve months many of the cows concerned have shown symptoms characteristic of the chronic forms of the disease.

Herd A is also notable for the relatively large number of severe cases of mastitis due to other causes, and especially to streptococci belonging to Group II, which have occurred. In this herd, contrary to the almost general rule, such infections have been the cause of much greater loss than mastitis of the common chronic type.

Herd C.

Herd C is made up of pedigree Jerseys and Red Polls, and from 1929, when observations commenced, it has carried over 100 head of milking stock. The herd has always been practically self-contained, though a few animals have been purchased from time to time. The herd is free from tuberculosis, certified milk being produced, and has been freed from contagious abortion by blood tests and segregation, coincidentally with the work reported here. Milking is carried out by hand.

Herd C.

Year.	*State of Herd at end of Year.*			*Additions, Removals and Cows which became Infected during previous 12 Months.*				
	Total.	*Healthy.*	*Infected.*	*Healthy became Infected.*	*Healthy Added.*	*Healthy Removed.*	*Infected Added.*	*Infected Removed.*
1929	86	53	34 (39·5)	—	—	—	–	—
1930	101	72	29 (28·7)	10 (29·4)	55	26	3	18
1931	86	61	25 (29·1)	4 (5·6)	10	17	0	8
1932	111	62	49 (44·1)	35 (57·4)	43	7	4	15
1933	113	68	45 (39·8)	11 (17·7)	25	8	2	17
1934	116	75	41 (35·3)	12 (17·6)	31	12	0	16
1935	134	99	35 (26·1)	7 (9·3)	39	8	0	13

For explanation see table for Herd A.

Of the 35 cows classed as infected in 1935, 11 had been free from infection for at least two years. The number of cows actually infected at the end of 1935 is, therefore, 24 and the real incidence 17·9 per cent.

Up to the end of 1931 mastitis-infected cows were kept in the same sheds as the uninfected, but segregated at one end and milked last. From 1932 onwards infected animals were removed to other sheds and milked by separate attendants. During the first four years heifers were milked after the older healthy animals until they had passed a test seven to ten days after calving. Since the middle of 1932 they have been milked first of all. In addition, the owner has always insisted that milkers should wash their hands after milking each cow and all reasonable precautions were taken to prevent spread from infected to healthy animals.

Complete herd tests of all animals in milk were carried out every three months in the case of the healthy group and at six- to twelve-monthly intervals in the infected group. Samples were

also examined seven to ten days after calving, and from any animal whose milk or udder became abnormal during the interval between herd tests. The total number of animals in the healthy section of the herd in December, 1929, or which have since entered this section ("Healthy added") is 256. Of these 256 cows, 72 were under observation for one lactation or less, 74 for two lactations, 47 for three, 32 for four, 24 for five and 7 for six lactations. The number of tests made of each cow was from 2 to 17 and in one case 24, a total of 1,621 or an average for each animal of between 6 and 7. The table shows the number of healthy and infected animals in the herd at the end of each year and the movements during the preceding twelve months.

The clinical severity of the disease originally present may be gathered from the following statements. By the end of 1929 all the original milking herd had been tested, except four cows which were still dry. Of the 34 infected cows present at that time, 17 were showing clinical symptoms or obviously altered milk in one or more quarters; 8 showed abnormality of the milk only recognisable by laboratory examination, whilst in 9 the milk was unchanged. The average number of infected quarters per animal was 2·4.

Comment.

In this herd no special attempt was made to dispose of infected cows, but they were immediately segregated, and what were expected to be ample precautions were taken to prevent spread of infection. Although there has always been a large group of infected cows on the farm in sheds adjoining those kept for healthy cows, it is somewhat surprising that new infections have continued to appear. Thus, of the 256 animals which were passed as healthy when first tested in the period 1929-35, 79 had become infected up to the end of 1935. Although dropping to less than 6 per cent. in 1931 the annual percentage of new infections rose suddenly to over 50 in 1932. It dropped again to 17 per cent. in 1933 and 1934, whilst in 1935 9 per cent. have occurred. The result is that the section described as infected, which was originally 39 per cent. of the herd, still forms 26 per cent. of it. As in Herd A a number of animals apparently recovered. These were, in nearly all cases, animals in which the infection was discovered only by means of the glucose broth enrichment medium and on only one occasion, in spite of numerous examinations during the succeeding years. Since such cows have always been classed as infected the number of animals shown as infected is, in this herd also, unduly high for all years since 1932. The real incidence at the end of 1934 was 17·9 per cent.

As in other herds, a certain amount of trouble due to mastitis streptococci of Groups II and III and to *C. pyogenes* has occurred, 18 cows in all being affected.

Herd I.

At the end of 1930, when the first examinations were completed, Herd I consisted of about 160 head of pedigree milking stock of the British Friesian and Shorthorn breeds. It has been maintained since that time solely by means of home-bred animals. The herd is tuberculin-tested and it has been freed from contagious abortion coincidently with the work reported here. Milking is carried out by machine three times a day and all cows are stripped by hand.

After the first herd test infected animals were segregated as far as possible, and milked last. In 1931 the cows were arranged on three standings of which one contained only healthy cows, one healthy and infected at opposite ends, and one infected cows only, one man doing all the work on each line. Three milking machine units were in use in each row, separate units being reserved for the infected cows in the composite line. Teat cups were sterilised at the beginning of milking but not subsequently unless they were dropped. Towards the end of 1931 the owner decided to reduce his herd considerably and amongst those sold were most of the infected cows. From March, 1932, the few remaining infected cows were moved to other buildings.

Herd I.

Year.	*State of Herd at end of Year.*			*Additions, Removals and Cows which became Infected during the previous 12 Months.*				
	Total.	*Healthy.*	*Infected.*	*Healthy became Infected*	*Healthy Added.*	*Healthy Removed.*	*Infected Added.*	*Infected Removed.*
1930	158	71	87 (55·1)	—	—	—	—	—
1931	125	76	49 (39·2)	16 (22·5)	60	39	7	61
1932	65	61	4 (6·2)	9 (11·8)	37	43	2	56
1933	61	61	0	0	31	31	0	4
1934	81	81	0	0	34	14	0	0
1935	79	79	0	0	18	20	0	0

For explanation see table for Herd A.

Milk samples were examined every three months. Samples were also taken from individuals cows seven to ten days after calving, and from any animal which was giving altered milk or which showed any clinical abnormality of the udder or reacted to tests with brom-cresol-purple paper which were regularly carried out at the farm. The total number of animals passed into the healthy section of the herd in 1930 or which have since entered this section (" Healthy added ") is 251. Of these 251, 118 were under observation for one lactation or less; 96 for two lactations; 22 for three; 10 for four, and 5 for five lactations. The number of tests made of each cow was from 2 to 22, totalling 1,476 or an average of about 6 for each cow.

The table, giving the changes in the herd year by year and its composition at December 31st of each year, shows that at the end of the third year the herd was free from *Str. agalactiæ* and has remained so since that time.

The mastitis originally present was of the characteristic chronic variety caused by *Str. agalactiæ*. The following data regarding the changes observed on one or more occasions will give some idea of its severity. Of the 96 cows of the original herd which were infected at their first examination in 1930 to 1932, 30 showed at or about the time of test clinical symptoms and obviously abnormal milk, 54 showed definite laboratory evidence of mastitis, whilst in 12 the milk was unaltered. The average number of infected quarters per cow was 2·6.

Comment.

In Herd I the contagious form of streptococcus mastitis was eradicated within a reasonable period in spite of the high original incidence (55 per cent.) and the fact that for the first 18 months infected cows were housed in the same shed as the healthy. It is to be noted, however, that during the first 18 months many animals in the healthy group showed new infections, *viz.*, 22 per cent. in 1931 and 12 per cent. in 1932, a circumstance which there is reason to believe may have been due in some measure to slackness on the farm. There is no doubt, of course, that the eradication of the disease from this herd was facilitated very considerably by the decision of the owner in the autumn of 1931 to reduce his stock. Most of the infected cows were sold then or during the first half of 1932, and other infected cows were from that time completely separated.

Apart from mastitis due to *C. pyogenes*, which has caused the loss of a small number of quarters, the herd has been strikingly free from obvious udder disturbance due to streptococci other than *Str. agalactiæ* and during the last three years there has been practically no mastitis of any kind.

Herd J.

This herd belonged to the same owner as Herds K and L, to be described next. Infected animals from Herds J and K were sent to other premises for isolation. There has been some interchange between the animals of Herds J and K, although different attendants were used on the two farms. Early in 1933 it was decided to raise a new herd on another farm, starting with freshly calved heifers. This is designated Herd L.

Herd J consists of about 45 animals of the Ayrshire breed and is maintained entirely by home-bred stock. It is free from tuberculosis; the eradication of contagious abortion was begun in 1932, and at recent tests there were no reactors. Milking is carried out by machine and all cows are stripped by hand.

The first tests for mastitis were carried out in November, 1931, and by the end of 1932 55 animals of the original herd had been tested for the first time. Of these 55 animals, 16 (29·1 per cent.) were infected.

All animals in milk were examined at intervals of three months during 1932 and subsequently at six-monthly intervals. All found to be infected at the first or later tests were removed as soon as convenient to the owner. Post-calving samples were not examined and animals found to be infected at any given test had, therefore, already been in the herd for some time. As mentioned, up to the end of 1932, 16 animals were recognised as infected at their first tests; in 1933 one such animal was located. The total number of animals classed as healthy at their first test in 1931 or subsequently was 92. Of these 92, 16 were under observation for one lactation or less, 32 for two, 20 for three and 24 for four lactations. The number of examinations made of each cow was from 2 to 11, a total of 464 or an average of about 5 for each animal. The table shows the number of animals in the herd at the end of each year and the movements during the previous twelve months.

The general character of the mastitis originally present, which was almost solely of the common chronic type, can be gathered from the fact that of the 16 cows of the original herd found to be infected, 4 showed at or about the time of test clinical symptoms or marked change in the milk, 9 showed definite laboratory evidence of mastitis, whilst in 3 the milk was unchanged in any way. The average number of infected quarters was 2.

HERD J.

Year.	*State of Herd at end of each Year.*	*Additions, Removals and Cows which became Infected during previous 12 Months.*		
		Healthy became Infected.	*Healthy Added.*	*Healthy Removed.*
1931	30	—	—	—
1932	31	6 (20·0)	9	2
1933	55	3 (9·7)	34	7
1934	45	5 (9·1)	8	13
1935	45	3 (7·0)	11	8

For explanation see table for Herd A.

Comment.

Although originally nearly one-third of the cows in this herd were infected, it is somewhat surprising that the infection has not been eradicated, since infected cows were removed to another farm. It is encouraging, however, that, whilst the number of animals in the herd has increased, the proportion of new infections dropped to less than 10 per cent. in 1933 and 1934, and to 7·0 per cent. in 1935. Failure to eradicate the disease may have been due to the relatively long intervals between samplings and because newly

added cows—many of which were infected—were not examined until the next herd test; also because cows found to be infected were sometimes not removed for weeks or months. With one exception the herd has been free from mastitis due to causes other than *Str. agalactiæ*, whilst obvious mastitis due to this organism has disappeared, such infections as have occurred being detectable by laboratory methods only.

Herd K.

This herd now consists of about 50 animals of the Friesian, Ayrshire and Guernsey breeds. It is maintained solely by home-bred stock, and is free from tuberculosis. The eradication of contagious abortion was begun in 1932 and the herd is now free from reactors. Milking is carried out by hand.

Mastitis control was begun in November, 1931, and by the end of 1932 93 animals of the original herd had been examined for the first time. Of these 93 animals, 50 (53·8 per cent.) were infected.

Herd K.

Year.	*State of Herd at end of each Year.*	*Additions, Removals and Cows which became Infected during previous 12 Months.*		
		Healthy became Infected.	*Healthy Added.*	*Healthy Removed.*
1931	36	—	—	—
1932	27	16 (44·4)	7	0
1933	40	9 (33·3)	31	9
1934	55	4 (10·0)	30	11
1935	49	4 (7·8)	21	23

For explanation see table for Herd A.

All animals in milk were examined at intervals of three months during 1932 and subsequently at six-monthly intervals, those found to be infected at the first or subsequent tests being removed as soon as convenient to the owner. Post-calving samples were not examined and animals found to be infected at any given test had, therefore, been in the herd for some time. As already stated, 50 animals were found to be infected at their first test up to the end of 1932; in 1933 and 1934 the corresponding numbers were 7 and 3. The total number of animals classed as healthy at their first test in 1931 or subsequently was 125. Of these 125, 46 were under observation for one lactation or less, 39 for two lactations, 30 for three and 10 for four lactations. The number of examinations of each cow was from 2 to 10, a total of 480 or an average of nearly 4 for each animal. The table shows the number of animals in the herd at the end of each year and the events during the previous twelve months.

The general character of the mastitis originally present, which was almost solely of the common chronic type, can be gathered

from the following data: of the 50 cows of the original herd found to be infected 8 showed, at or about the time of test, clinical symptoms or marked naked-eye changes in the milk, 31 showed definite laboratory evidence of mastitis, whilst in 11 the milk was unchanged in any way. The average number of infected quarters was 2·6.

Comment.

Although, since infected cows were removed, it might perhaps have been expected that the disease would by now have been eradicated, the fact that the number of infections has steadily fallen whilst the herd has been increasing is satisfactory. Thus, whereas the original percentage of infection was over 50 per cent., the percentages of new infections in 1932, 1933, 1934 and 1935 were 44, 33, 10 and 7·8 respectively. Failure to eradicate the disease may have been due to the same factors as operated in Herd J. The herd has been entirely free from mastitis due to causes other than *Str. agalactiæ*, whilst mastitis due to this organism has been confined to latent cases.

Herd L.

This herd was formed early in 1933 from freshly calved Ayrshire heifers belonging to the owner of Herds J and K. It is free from tuberculosis and contagious abortion. The animals are hand-milked.

Tests were carried out at intervals of six months and any infected animals at once removed. Post-calving samples were not taken. The total number of animals which have come under observation is 48, of which 13 have been in the herd for one lactation or less, 16 for two and 19 for three. During this period they have been examined 2 to 9 times—a total of 266 examinations or an average of between 5 and 6 for each cow.

The state of the herd in February of 1933 and at the end of each year and the events during the preceding twelve months are shown in the table.

Herd L.

Year.	*State of Herd at end of each Year.*	*Additions, Removals and Cows which became Infected during previous 12 Months.*		
		Healthy became Infected.	*Healthy Added.*	*Healthy Removed.*
1933 (Feb.)	22	—	—	—
1933	32	1	12	1
1934	30	1	6	7
1935	31	0	8	7

For explanation see table for Herd A.

Comment.

Of the 23 heifers drafted to this herd at the beginning of 1933, one was found infected at the first test. Twenty-six other heifers have been added and up to the time of this report two have been found to be infected. Although it was hoped that no infection at all would appear in this herd, the results are satisfactory so far as they go and show that when newly-calved heifers are kept apart from older cows the spread of *Str. agalactiæ* can be prevented.

Apart from one heifer which showed some disturbance in two quarters, due to Group III streptococci, the herd has been free from udder disturbance of any kind.

The Incidence of Infection in Cows of Different Ages.

In Table 6 is given a summary of the data obtained at the beginning of our work in Herds C, I, J and K, to show the incidence of infection in cows of different ages. In these herds the original incidence of infection was 36·2, 55·1, 29·1 and 53·8 per cent. respectively. Whilst the actual figures for age incidence vary somewhat in the individual herds, there is a gradual increase of infection with succeeding lactations, and it is seen that unless precautions are taken to protect heifers from infection as many as 30 per cent. may become infected in their first lactation. These findings are similar to those of others, *e.g.*, Seelemann (1932).

Table 6

Incidence of *Str. agalactiae* in Relation to the Number of Lactations.

Herd.	*1st Lactation.*			*2nd Lactation.*		
	Total.	*Number affected.*	*Per cent.*	*Total.*	*Number affected.*	*Per cent.*
C	34	2	5·9	23	7	30·4
I	54	14	25·9	61	38	62·3
J	17	1	5·9	7	1	14·3
K	20	6	30·0	12	3	25·0
Totals	125	23	18·4	103	49	47·6

Herd.	*3rd Lactation.*			*4th to 10th Lactations.*		
	Total.	*Number affected.*	*Per cent.*	*Total.*	*Number affected.*	*Per cent.*
C	14	8	57·1	23	17	73·9
I	32	23	71·9	11	11	100·0
J	8	2	25·0	23	12	52·2
K	11	9	81·8	50	32	64·0
Totals	65	42	64·6	107	72	67·3

Discussion.

In 1933, Minett, Stableforth and Edwards demonstrated the possibility of assembling a herd of cows free from *Str. agalactiæ* infection. The initial incidence of infection in the herd concerned

(Herd B), which contained 49 cows, was 24·5 per cent., and in 1933 infection had been absent for about three-and-a-half years. At the present date the herd is still free.

In 1933 control work was also proceeding in the six herds now being discussed. It was hoped that by the use of similar measures it would be possible to eradicate the disease from these herds or, at least, to reduce the number of new infections to such a point that, with the normal disposal of animals as they got older, final elimination would be in sight.

In Herd I our hopes have been realised, since it has now been free from infection for nearly three years. Herds A. C. J and K, on the other hand, have continued to show new infections, although in all four herds the proportion has steadily fallen whilst the size of the herds has increased. With Herds J and K a number of circumstances have probably contributed to the failure to effect eradication. Thus, the intervals between herd tests had to be prolonged to about six months, post-calving samples were not examined, and freshly drafted cows were not examined at once, and, finally, it was impossible in some cases to remove infected animals to the separate farm for some weeks or months.

With Herds A and C there were several factors which increased the difficulties of control. Firstly, although the owners had prescribed strict precautionary measures for preventing spread of disease, a large infected section had to be maintained in the same or neighbouring sheds. Although it is difficult to see how the precautions taken could have been improved, the results, in conjunction with those of Herd I, suggest that the procedure of keeping infected and non-infected cows in the same or adjoining sheds is less satisfactory than the total removal of infected cows. This accords with the view of Seelemann (1933), that unless infected cows can be quickly evacuated the removal of infection by suitable treatment is in most cases a valuable and perhaps essential aid to eradication. Secondly, in both herds the retention of *Str. agalactiæ* infected cows has helped to keep up the size of the infected group. Thus, in Herd A, there has been a large number of cases of clinically evident mastitis due to streptococci other than *Str. agalactiæ*, apparently arising in a sporadic manner. The *Str. agalactiæ* infections, on the other hand, were insignificant clinically, and it was not to be expected, therefore, that the owner would evacuate these in preference to the more obviously affected animals. In Herd C it is to be noted that many infected cows were retained much longer than they would have been under ordinary circumstances owing to their high pedigree breeding value.

In Herds A and C the results at first sight appear to be disappointing. When the data are analysed, however, the results are less discouraging than they appear, for the following reasons. It should be noted that in 1932 the laboratory control was made

more strict and, also, that a selective enrichment medium was introduced, capable of detecting very small numbers of streptococci in milk. The considerable increase in the number of new infections revealed in Herds A and C in 1932 may thus be accounted for. It follows at the same time that the incidence of infection prior to 1932 was probably higher than is represented by the figures given. Subsequent to 1932 the number of new infections fell, although not so much as was anticipated. In both herds a large number of new infections—mostly with non-hæmolytic *Str. agalactiæ*—were diagnosed solely by means of the enrichment medium and such animals were practically always giving normal milk. In a few cases chronic infections developed later or an obvious mastitis associated with large numbers of streptococci, but in nearly all cases infection was shown at one test and not at subsequent tests. In practice, however, such cows were always treated as infected and they appear as such in the tables, so long as they were still present in the herd. Thus, although the infected sections appear large in these two herds, many of the cows therein are not in fact infected at the present date. On the other hand, most of the cases which were diagnosed by direct plating of milk deposits have shown persisting infections. It should be said, finally, that the streptococci concerned in the transient infections mentioned were typical *Str. agalactiæ* biochemically and serologically and in some instances have been found capable of producing a chronic infection when injected into the udder of goats experimentally *via* the teat canal.

The experience in Herds A and C is of particular interest in establishing the fact that *Str. agalactiæ* is capable of setting up transient self-healing infections which can only be detected by the most stringent bacteriological tests. Since most of such infections were diagnosed by the enrichment medium only, there is some reason for regarding the use of an enrichment medium as superfluous for the control of the disease in practice.

Herd L remains for comment. This, it will be remembered, was formed from freshly-calved heifers and hence was expected to remain entirely free from *Str. agalactiæ*. As already stated, however, one heifer was found to be infected at her first test and two others subsequently became infected. Nevertheless, the disease is well under control and, although it is still too soon to conclude that infection will not appear later, the incidence of infection amongst these animals, most of which are now in their second or third lactation, need only be compared with that shown in Table 6 to realise that where chronic mastitis is at all prevalent the formation of a separate heifer herd is a measure of the greatest value. Emphasis has been laid in another article (Stableforth, 1935) on the practicability of forming a separate herd from young animals and the value of this procedure in protecting them from infection with other chronic contagious diseases besides mastitis.

Summary and Conclusions.

An account is given of attempts in six herds to control chronic mastitis due to *Str. agalactiæ*. Latent infections were diagnosed by making cultural tests of the milk in blood agar plates or in a selective enrichment medium, these methods being supplemented by taking the chemical reaction of the milk and by noting the amount and character of the centrifuge sediment. These tests were made at three- to six-monthly intervals and in three herds also shortly after calving. Infected animals were milked after the healthy ones either in the same shed or in another shed on the same premises, or they were removed to another farm and milked by different attendants.

One herd (I) in which the original incidence of infection was 55 per cent. has now been free for three years, a contributing result being the early sale of most of the infected cows. In three others (C, J and K) in which the original incidence was 40, 30 and 53 per cent. respectively, the number of new infections has decreased during four to five years to 9·3, 7 and 7·8 per cent. respectively in 1935. Herd L under the same ownership as Herds J and K was built up early in 1933 from freshly-calved heifers and is at present free. In Herd A the incidence and numbers of new infections were at first low; the number of new infections rose in 1932, but since then has steadily fallen to 5·3 per cent. in 1935.

The results are discussed in the light of the conditions prevailing in each herd and of the degree of control that could be exercised. For instance, in Herds J and K, although a separate isolation farm was used, the interval between tests could not be less than six months. In Herds A and C infected cows were kept in the same shed as the healthy or in sheds on the same premises, but in both herds, for reasons given, infected cows were retained far longer than would normally have been the case. Actually in Herds J, K, A and C the results are more encouraging than they appear. Thus, in general the new infections due to *Str. agalactiæ* have been insignificant clinically. Also, the real incidence of infection is at present less than the above statement simply because many cows, in which infection was diagnosed only by means of the enrichment medium and on one occasion only, have now been free from infection for at least two years although in practice they are still regarded as infected.

The experience in the present herds and in Herd B of the previous paper (1933) justify the following conclusions:—

(1) Eradication of contagious streptococcus mastitis may be achieved under certain conditions by the simple expedient of segregating infected cows or, if that is not possible, by milking them last. The chances of success, however, are greatly increased if infected cows can be evacuated within a reasonable time. When

this cannot be done, infusion of the infected udder with a suitable bactericidal agent should prove a useful aid.

(2) Whilst *Str. agalactiæ* in nearly all cases leads to permanent infection, it occasionally causes transient infections detectable only by the use of enrichment media.

(3) Experience has shown that the disease does not arise sporadically once all known sources of infection are removed. New infections must, therefore, be due to errors in milking or to deficiencies in methods of diagnosis.

(4) The formation where possible of a separate heifer herd is a measure of the greatest value.

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TREATMENT OF TRYPANOSOMIASIS BY TARTAR EMETIC, ANTIMOSAN AND "SURFEN C" IN THE GOLD COAST

By J. L. Stewart, M.C., B.SC., M.R.C.V.S.

In view of the results of tartar emetic treatment for trypanosomiasis in Nigeria by Marshall, Lester and Jones, those obtained in cattle in the Gold Coast may be of interest.

The cattle of the Gold Coast are different from the bovines found in Nigeria. The latter are humped zebus while the former belong to the West African Shorthorn unhumped breed of cattle. Unquestionably the West African Shorthorn has a much higher resistance to trypanosomiasis than the zebu. In the Gold Coast humped cattle cannot thrive as they soon succumb to trypanosomiasis. The West African Shorthorn is the second oldest African bovine, the oldest being the Hamitic Longhorn, which may be regarded as the indigenous cattle beast of West Africa while the zebu is a much more recent import, having originally been brought from Asia with the Fulani, a tribe analogous to the Masai of East Africa. Trypanosomiasis causes few fatalities in the West African Shorthorn cattle of the Gold Coast though it is responsible for great loss of condition and stunting of size, and though deaths from trypanosomiasis are scanty the latter disease appears to act as a catalytic agent for other protozoal conditions. In areas where trypanosomiasis infection is heavy there is always much red-water, gall sickness and theileriasis of a pathogenic type. At immunisation camps, where over 170,000 cattle have been treated against rinderpest since 1930, the numbers of breakdowns to trypanosomiasis can be numbered on the fingers of one hand, while breakdowns to the enzoötic diseases mentioned have been considerable and responsible for most of the casualties at the camps. This state of high resistance to trypanosomiasis is common to the West African Shorthorn throughout West Africa, where that bovine exists in large numbers in French Senegal, Guinea, Ivory Coast, Dahomey, Togo and elsewhere. Some variations of the breed, such as the tawny N'Dama, are stated to possess even higher resistance than ordinary, a fact which appears credible as the result of filed observations and a few observed experiments at the Pong-Tamale laboratory. So susceptible to trypanosomiasis is the zebu that in the Gold Coast he is regarded as an exotic breed and crossing experiments have shown that zebu blood lowers resistance.

These few explanatory facts are necessary to qualify the results of treatment here compared with the facts noted in Nigeria. Another difference is that the cases treated here were all transmitted to the cattle by natural infection by *G. tachinoides*, with the possibility of a few being infected by *G. palpalis*, the proportion of

the latter fly to the former being 1 to 61 in 1933, or by direct infection by biting flies. It will be noted that some of the cattle infected were zebu or half-bred zebu-West African Shorthorn.

(1) Cattle infected by trypanosomiasis and treated by antimosan in 1933 in August to October inclusive.

Type.	*Trypanosome.*
West African Shorthorn 14 (10 cows, 4 calves).	One *T. congolense*, rest *T. vivax*.
Zebu 5 (3 cows, 2 heifers)	One mixed vivax and congolense, rest *T. vivax*.
¼ bred Hereford 2	*T. vivax*.
½ bred zebu-West African Shorthorn —8	All *T. vivax*.

Total—29 head.

Five injections of antimosan were given at weekly intervals. Repeated microscopic examinations have been made to date (May, 1935). There has been one relapse in a West African Shorthorn cow in December, 1933, which animal died after the second injection of tartar emetic, this drug being used instead of antimosan. One zebu cow has since died of turning sickness. The remaining 27 cattle have been negative since 1933, have all maintained good condition and have been normal in every way.

(2) Cattle infected by trypanosomiasis and treated by tartar emetic in 1933 between July and December.

Type.	*Trypanosome.*
West African Shorthorn 10, including 3 N'Dama bulls	2 *T. congolense*, rest *T. vivax*.
Zebu—2	*T. vivax*.
½ bred zebu—4...	*T. vivax*.

Total—16 head.

Treatment was by five intravenous injections at intervals of five days. All these cattle are still negative, with the exception of one West African Shorthorn cow, which has relapsed twice, once in 1933 and again in 1934, but has been negative since then.

(3) Cattle treated by one intramuscular injection of " Surfen C," a Bayer experimental product.

(*a*) In 1933.

Type.	*Trypanosome.*
West African Shorthorn—17 head, all bullocks	All *T. vivax*.

Five of these bullocks have been positive since, one of them on two occasions, but these five have been negative since end of 1934.

(*b*) In 1934, from April to September.

Type.	*Trypanosome.*
West African Shorthorn—31 bullocks	1 *T. theileri*, 1 *T. brucei*, 8 *T. congolense*, remainder *T. vivax*.
Zebu—7 bullocks	2 *T. congolense*, rest *T. vivax*.

Of these bullocks, four West African Shorthorn animals have broken down, two *T. congolense*, two *T. vivax*; five of the seven zebus have also relapsed, one to *T. congolense* and the other four to *T. vivax*. This treatment by " Surfen C " was experimental and does not appear to be nearly as effective as tartar emetic and other antimony preparations, though " Surfen C " appears to be useful after antimony treatment to prevent relapses.

All of these 85 cattle are alive save two animals only, which did not die from trypanosomiasis. The zebu bullocks which relapsed are in very bad condition and are being disposed of, but the zebu cows and heifers treated by tartar emetic and antimosan appear to have made a complete recovery and are in excellent condition now in mid-1935.

The bullocks were work oxen or serum animals, *i.e.*, used for the production of anti-rinderpest serum at the Pong-Tamale Laboratory.

Sodium antimony tartrate and not the potassium salt was used, as the former is considered less toxic. As far as curative results are concerned, there would appear to be nothing between the two salts.

Dosage of tartar emetic averaged 12 to 15 grains per adult. Antimosan was used in 40 c.c. doses to adults down to 5 grains in calves. " Surfen C " is used in 100 c.c. doses, 50 c.c. being given intramuscularly into the muscles of the shoulder and 50 c.c. into the muscles of the rump.

The domestic animal most susceptible to trypanosomiasis in the Northern Territories is the horse. Treatment of horses by tartar emetic has yielded good results over a period of some ten years. Horses can be kept under observation more generally than any other animal, and there are in Tamale and environs several horses which were treated over five years ago and have not relapsed. One horse which became infected by *T. vivax* in 1931 has apparently become " fast " as far as antimony is concerned. He has had eight tartar emetic courses and two antimosan and has also been treated with naganol, tryparsamide and " Surfen C." At present he is negative to blood examination, having just undergone a course of intravenous injections of 25 grains sodium tartar emetic each.

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REVIEWS

Brucella Infection in Swine. By Axel Thomsen, State Veterinary Serum Laboratory, Copenhagen. Levin and Munksgaard, Copenhagen, 1934. Translated from Danish by Hans Andersen, M.D. Price 16s.

This work has 242 Royal 8vo pages, of which approximately the first 50 give an outline of the different forms of brucellosis in man and the domestic animals. The remainder gives an account of the author's investigations in connection with a remarkable epizootic of brucellosis in swine which prevailed in Denmark in the period 1929-32.

Since the foundation of the State Veterinary Serum Laboratory in Denmark in 1910, under the Public Service diagnostic examinations have been carried out regarding contagious abortion, and during the first 20 years examination of suspected cases of the disease in swine always had a negative result. It was therefore with surprise that microscopic examination of the placenta of an apparently infected sow for the first time revealed the presence of typical clumps of bacilli. The suspicion thus excited was confirmed by an agglutination test. This was the starting point of the epizootic, and a detailed account is given of the investigations which were conducted regarding the disease in swine and of the organised efforts by which it was eradicated in 1932.

It was not possible to trace the origin of the disease at the places where it was first discovered, but there was evidence indicating that early in March, 1929, the disease had been spread extensively by the sale of pregnant sows from a particular centre. The infection in Jutland gradually involved a total of 183 swine herds, mostly of small size and comprising altogether 1,146 animals, and serological examination of these showed 23 reacting boars and 477 reacting sows and gilts. Spread of infection through the boars was a conspicuous feature of the epizootic. The most extensive infection transmitted by one boar involved 25 herds. Thorough and extensive investigations revealed with certainty how the infection was transferred to Zealand with a shipment of infected sows. There the infection spread through service to 57 herds comprising a total of 277 animals, among which 9 boars and 118 sows and gilts gave positive reactions for brucellosis. From April 1st, 1932, to April 1st, 1933, only 3 new herds were found to be infected, and the last case of the disease was ascertained on December 21st, 1932. With this the epizootic was eradicated in Denmark. The result was obtained by examination of 940 afterbirths from aborting sows and gilts, 6,941 samples of blood from herds suspected of infection, and 24,731 samples of blood from slaughter houses (sows and boars).

The origin of the epizootic is discussed but nothing definite with regard to that was discovered. It is pointed out that the epizootic was somewhat analogous to the appearance on a large scale in recent years of undulant fever in man in Denmark.

The description of the lesions found in infected sows and boars forms a most interesting part of the work. A common condition in infected sows was what the author terms " miliary brucellosis of the uterus." It appears in the form of numerous well-defined, whitish-yellow nodules, varying in size from those just visible up to those pea-sized. These lesions in the uterus are taken to be the primary changes in porcine brucellosis. Excellent photographs of them are given, and also of the lesions in boars.

The investigations revealed differences between the Danish porcine strain and bovine strains, and also minor differences between the Danish and the American porcine strains. The differences, however, were not

considered sufficient to exclude the possibility that the American *Brucella suis* had somehow been introduced into Denmark and given rise to the epizootic there.

The work is a masterly production, and will be welcomed everywhere as the most complete account of brucellosis in swine that has yet appeared in any language.

Localized Rarefying Conditions in Bone. By E. S. J. King, M.D., D.SC., F.R.C.S. London : Edwin Arnold and Co., 1935. Price 35s. Pp. 400 and 69 figures.

In this work the author deals with localized rarefying conditions in bone as exemplified by Perthes' disease, Osgood-Schlatter's disease, Kümmell's disease. The morbid conditions grouped under these names have yielded similar characters under röntgenological examination, but their cause or causes have not been determined. The material personally investigated by the author comprised 160 cases chosen from a large number encountered in hospital and private practice, and the illustrations have been taken from these.

The opening sections deal with the nature of bone—its origin and histological structure, its chemical composition, and its physiological activity, including calcification ; the bulk of the work treats of osteo-chondritis as it affects the different bones in the human subject. Thirty-two pages are devoted to the diseases named in the title of the book, which are mainly cases of juvenile " hip-joint disease " or osteo-chondritis of the upper epiphysis of the femur. The ætiology of the disease is discussed at length, and one learns that tuberculosis, syphilis, and the common pyogenic organisms have been incriminated, but that none of the views advanced has been generally accepted. (The bibliography of the disease covers 16 pages !) The present author is of the opinion that it is not a definite disease, but a syndrome of a number of clinical signs and symptoms with a characteristic röntgenological picture. This disappointing conclusion must be attributed to the chronic, usually non-fatal course of the disease, which greatly restricts the opportunities to study the progress of the alterations in the joint and the possible presence of bacteria.

A study of the book would be a necessary preparation for any further investigation of the disease in question, or of similar joint diseases in animals.

The Internal Parasites of Domestic Animals. A Manual for Veterinary Surgeons. By Thomas W. M. Cameron, T.D., M.A., PH.D., D.SC., M.R.C.V.S. Research Professor of Parasitology, McGill University, Canada. London : A. and C. Black, Ltd. 1934. Price 15s. net.

This work extends to 284 pages, and after an introduction of 18 pages, including nomenclature and history, the Protozoa are dealt with in 44 pages and the Helminths in 174 pages. The remaining 70 pages deal with Immunity and Serology, Therapeutics of Helminthic Infections, and Technique.

There are 144 figures in the text, and one coloured plate.

On the title page the book is described as a Manual for Veterinary Surgeons, but one learns from the preface that the author's ambition was to produce " a text-book on Parasitology, useful alike to practitioner and student," which, he thought, had been long overdue.

The author's conception of the information on parasitology that ought to be provided in a book for veterinary surgeons appears to have been at fault. It is true that it is replete with illustrations and diagrams, but the

text is in many places very thin in regard to the information which is likely to be of most service to the veterinary surgeon in actual practice.

In the Bibliography of seven pages at the end of the book it is impossible to recognise on what principle the works mentioned in it were chosen, but some of the authors not included are made very prominent by their omission.

Chirurgische Operationstechnik. By Dr. Oskar Roder und Dr. Ewald Berge. Third Edition. Berlin : Paul Parey. 1935. Price, bound, 8.60 Rm.

In the new edition of this very practical guide to surgical operations the text has been revised and brought up to date by the inclusion of operations recently introduced and the addition of a number of illustrations. Naturally, it deals mainly with operations on the horse, but it also includes those of any importance in the other domesticated animals. Casting and other methods of restraint and anæsthetics occupy the first 30 pages, and the following sections deal in succession with the operations on the different parts of the body. The illustrations, now numbering 124, and many of them coloured, add greatly to the practical value of the work. German students are to be congratulated on having such an excellent guide to their exercises in surgery.

The Physiology of Domestic Animals. By H. H. Dukes, D.V.M., M.S., Professor of Veterinary Physiology, New York State Veterinary College, Cornell University. Third Edition. Ithaca, New York, Comstock Publishing Company, Inc. 1935. Price 6 dollars.

There is probably no branch of a veterinary student's professional education that is more important than physiology, and if the subject is to be well taught the student must have the aid of a good text-book. The present work deserves to be so described. It is thoroughly up-to-date, the space devoted to each part of the subject is adequate to its importance, and the text is clear and concise. It can be strongly recommended both to teachers and students.

The Essentials of Histology. By Sir E. Sharpey-Schafer, F.R.S. Thirteenth Edition. Edited by H. M. Carleton, M.A., B.SC., D.PHIL. London : Longmans, Green and Co. 1934. Price 15s. net.

The call for a new edition of this standard work is evidence that it maintains the high place which it has long occupied as a guide to the study of histology. It extends to 605 pages, in which there are 721 illustrations, many of them coloured. It is hardly an exaggeration to say that nothing relating to the histology of the organs and tissues of the body is left to the student's imagination ; all are presented to the eye with clearness and accuracy. It is, of course, primarily designed for medical students, but advanced veterinary students will also find it almost equally useful.

Topographical Anatomy of the Dog. By O. Charnock Bradley, M.D., D.SC., F.R.C.V.S. Third Edition. Edinburgh : Oliver and Boyd. 1935. Price 25s. net.

The new edition has been increased by a few pages, and it contains a number of new illustrations. These now number 80, and in them all the different parts of the body are displayed in their proper relationships.

To praise the book would be a work of supererogation ; it remains unrivalled as a guide to the dissection of the dog.